

TX 612
.S4 C6
Copy 1

The University of Washington
Department of Chemistry

**A Biochemical Study of Pacific Coast Salmon
with Particular Reference to the Formation
of Indol and Skatol During Decomposition**

by

RAY WILLIAM CLOUGH

**A Thesis Submitted in Partial Fulfilment of the Requirements for
the Degree of Doctor of Philosophy**

Seattle
University Press, University of Washington
1922

LIBRARY OF CONGRESS
RECEIVED
MAY 9 1923
DOCUMENTS DIVISION

31

**A Biochemical Study of Pacific Coast Salmon With Particular
Reference to the Formation of Indol and Skatol
During Decomposition¹**

RAY WILLIAM CLOUGH²
University of Washington

1. The salmon canning industry.....	196
2. Examination of commercial canned salmon.....	200
A. Systematic method	200
3. Chemical composition of fish flesh.....	204
4. Decomposition of fish flesh.....	209
5. Development of a method for detecting decomposition by means of indol and skatol.....	214
A. Selection of suitable color tests.....	214
B. Modification of the selected color tests.....	217
C. Distillation of indol from salmon.....	219
D. Extraction of indol from the distillate.....	221
E. Evaporation of the ether extract.....	222
F. Color tests on the water test solution.....	223
G. The method as finally developed.....	224
6. Experimental work on the five species of salmon in different stages of decomposition.....	225
A. General outline of the method employed.....	225
B. Experimental work on king salmon.....	229
C. Experimental work on pink salmon.....	230
D. Experimental work on sockeye salmon.....	235
E. Experimental work on coho salmon.....	235
F. Experimental work on chum salmon.....	240
G. Discussion of the results.....	246
7. Formation of indol by various means.....	253
A. Indol formation by bacteria.....	253
B. Formation of indol by scorching proteins.....	256
C. Formation of indol during the processing of salmon.....	257
D. Effect of exhaust on the indol content of canned salmon.....	258
8. Other decomposition changes.....	259
A. Volatile nitrogen as a measure of decomposition.....	259
B. Increase in free fatty acids as a measure of decomposition in salmon.....	263
C. Formation of a substance having a biting taste.....	265
9. Summary	265
10. Bibliography	268

¹ This investigation was made at the University of Washington in connection with other problems affecting the salmon canning industry.

² The writer is deeply indebted to Dean C. W. Johnson of the University of Washington, and to Dr. E. D. Clark, Dr. C. R. Fellers, Mr. O. E. Shostrom and Miss M. G. Haslam of the National Cannery Association, for advice, encouragement and help during the course of the investigation.

1. THE SALMON CANNING INDUSTRY

The value and importance of the salmon canning industry will be realized from a study of the following brief table summarizing the number of cases of salmon packed since the inception of salmon canning on the Pacific Coast in 1864. This table gives the total cases packed for the whole Pacific Coast, including British Columbia, and, for the sake of brevity, is given in ten-year periods, with the exception of the first period which covers only eight years.

TABLE 1. *Volume of canned salmon since the inception of the industry.*

	Period	Cases ³
	1864-1871	504,000
	1872-1881	5,013,861
	1882-1891	11,709,915
	1892-1901	26,864,515
	1902-1911	43,244,258
	1912-1921	73,377,223
Total	1864-1921	160,713,772

In addition to the salmon which are canned, millions of salmon are preserved each year in various other ways, such as drying, salting and freezing. However, more are canned than are preserved in all other ways combined.

Five species of salmon are found in the Pacific Ocean, all belonging to the genus *Oncorhynchus*. The Atlantic salmon belongs to a distinctly different genus, the genus *Salmo*. The Pacific salmon are found along the whole north semicircle of coast from central California to central Japan, and some of them have been successfully introduced into the southern hemisphere, particularly in New Zealand. Each of the five species is known by its scientific name, and by several common names, as shown in table 2.

These five species vary greatly in size, ranging from 3 pound (1.4 kg) pinks and sockeyes to 100 pound (45 kg) kings. The average weight is about as follows: Pinks 5 pounds (2.3 kg), sockeyes 5 (2.3 kg), chums 9 (4 kg), medium reds 10 (4.5 kg), and kings 30 (13.6 kg). They vary somewhat in habit and life history but all are alike in one essential particular,—they are anadromous, that is, when they reach maturity each species comes surging in from the sea to some fresh water stream or lake to spawn, after which practically all

³Reduced to a common basis of 48 one pound (454g.) cans to the case. From data given by Cobb (1921).

TABLE 2. *Names of the five species of salmon.*

Scientific name	Puget Sound	Columbia River	Alaska	Other names
<i>Oncorhynchus nerka</i>	Sockeye	Blueback	Alaska Red (Sockeye)	Quinault Redfish
<i>Oncorhynchus tshawytscha</i>	Spring	Chinook	King (Chinook)	Tyee, Quinnat
<i>Oncorhynchus kisutch</i>	Coho Silver	Silver	Medium Red, Coho	Silversides
<i>Oncorhynchus gorbuscha</i>	Pink	Pink	Pink	Humpback
<i>Oncorhynchus keta</i>	Chum	Chum	Chum	Keta, Dog, Calico

of them die and thus complete their life history. The fertilized eggs after an interval depending upon many factors, hatch, and the young fish spend a certain amount of time in fresh water before going to their ocean home.

The salmon industry depends to a great extent upon this spawning migration, for it is only at this time that the salmon may be caught in quantity, and indeed three of the species are almost never caught at any other time. Coming in from the open sea in immense schools they fall an easy prey to various types of nets, to traps and even to hook and line. The fish caught in nets or by hook and line (trolling) are taken into the fishermen's boats and often are transferred to cannery tenders. After reaching the cannery they are unloaded either by sluicing with water into elevators or by pitching with one-tined forks called pughs.

A few of the canneries dress or "butcher" the fish by hand but in most plants a machine known as the "iron chink" is used. This machine removes the head, tail, fins and viscera, and cleans the body cavity by means of brushes and jets of water. The salmon are then "slimed" by hand or by machine. In this process the blood, slime, loose membranes, etc., are removed with knives, spoons, or machines consisting of brushes revolving under jets of water. The cleaned fish are then cut into slices suitable for the different sized cans, either by a rotary hand-cutter or by "gang knives" consisting of revolving disks. The cans, already salted to the extent of $\frac{1}{4}$ ounce (7.1 g) of dry salt per 1 pound (454 g) can, are filled by hand or machine. A recent type of filling machine cuts the fish into slices, salts the cans and fills them at the rate of 115 to 125 per minute. Many of the flat cans are filled by hand; this is particularly true of the chinook

and sockeye salmon. The filled cans are often weighed by hand or machine, and are inspected to see that they have no bones or skin showing on top.

After filling, the cans are usually run through an "exhaust box" filled with live steam, in which the contents of the cans becomes heated and the air is replaced by steam to a certain extent. This may be done before the tops are placed on the cans, but in most canneries the top is first lightly clinched on by machinery to prevent particles of fish from getting out and water of condensation from getting in. In a few canneries the cans are exhausted by air pumps in the "vacuum closing machine." The primary object of exhausting the cans is to produce a vacuum which will keep the ends concave under changing conditions of temperature and altitude. The hot cans from the exhaust pass immediately to the "closing machine" (or "double seamer") which rolls the tops on very firmly, making the cans air tight. The cans, still hot, are placed in trays ("coolers"), stacked on a car and the car pushed along a track into a retort for cooking with live steam. Pound (454 g) cans of salmon are cooked ("processed") usually for 90 minutes at 117° C. (242° F.) or over; half pound (227 g) cans a few minutes less. The processing has several objects; to cook the fish and make it palatable, to soften the bones, and to render it sterile by killing all living organisms within the can. The pre-heating received in the "exhaust box" materially shortens the time necessary for sterilization.

After processing, the cans are run through a "lye bath" to remove oil and grease and are then thoroughly washed with water and set aside to cool. Leaky or defective cans are detected by tapping the bulging ends with a spike or metal rod; the difference in sound is a sure test for leaks. After cooling, the cans may be lacquered and either stacked in piles, or immediately labeled and placed in cases of 48 cans each.

Fish are very delicate, easy to injure and very easily decomposed. They must be handled as expeditiously as possible and should be in the cans within 48 hours after they are drawn from the water. There are many opportunities for spoilage between the time of catching and of canning. Salmon caught in gill nets soon suffocate, and if not promptly taken from the nets will become somewhat soft. They may be left in small boats for a day or more before the boat arrives at the cannery or at the cannery tender.

The fish are frequently transferred from one boat to another, to the tender and to the cannery by pitching with one-tined forks. Each

time they are thus pugged, bacteria are introduced deeply into the hitherto sterile flesh, and from these centers of infection the bacteria rapidly penetrate in every direction.

Salmon caught by seines are hauled into the boats alive but are frequently placed in deep holds to such a depth that the bottom fish are badly crushed and softened, and spoil very rapidly, particularly since the holds are usually warm and unventilated. A long haul to the cannery under such conditions results in considerable spoilage. A better practice is to place the fish in bins either in the hold or on the deck to a depth of only two feet (61 cm). Separating the fish in bins reduces the sliding about and bruising. If the fish are placed on the deck they should be covered with boards or tarpaulins as a protection from the sun.

Salmon caught by hook and line as in trolling are usually fish which are still feeding, that is, they are either not very far advanced on the spawning migration or are immature; mature salmon, reaching brackish or fresh water, seldom eat anything more, and thus the digestive tract is usually empty and free from bacteria. Fish caught by trolling, with food in them, deteriorate very rapidly; the bellies in time becoming so soft that they break through, a condition known as "belly-burning." A peculiar thing about such fish is that although their appearance may be very poor they may have no odor of decomposition, and in frequent chemical tests we found neither indol nor skatol. It is probable that the softening and the breaking down of the tissues is due not to bacterial decomposition but to the action of the enzymes in the digestive juices. Salmon caught by trolling should therefore be cleaned at once and if possible placed on ice.

Salmon caught in traps are brailed alive into boats or scows and should reach the cannery in good condition if the traps are not located too far away and if the boats are not delayed by bad weather. Even if the fish reach the cannery in first class condition there may be a considerable delay due to an over supply or to a break-down in the machinery. It is apparent, therefore, that in handling so perishable a commodity as fish there are many opportunities for spoilage, and the excellent condition in which the bulk of the canned salmon reaches the market speaks well for the care and resourcefulness of the average packer.

All of the conditions and practices which I have mentioned leave their mark upon the fish, and this record may be read in the canned product by the experienced examiner.

2. EXAMINATION OF COMMERCIAL CANNED SALMON

After the salmon has been canned it is very likely to be examined at least once before reaching the consumer. Brokers may buy a parcel of several thousand cases on the reputation of the packer but they are more likely to stipulate that the parcel be examined either by themselves or by some one in whom they have confidence. Little or no attempt has been made to grade canned salmon as is done with butter, cheese and many other foodstuffs. The only grading has been by the species and the district where packed. This is very unsatisfactory. If grading is ever placed on a scientific basis it will be by means of carefully kept records covering all species and districts. To obtain these records a systematic method of examination covering everything which is significant regarding the workmanship on the parcel and regarding the quality and condition of the fish must be followed. Partly as the result of the study of the five species of salmon, which is described in the experimental part of this thesis, and partly as a result of the careful examination of several thousand cans of commercially canned salmon, the following systematic method for the examination of canned salmon was evolved.

A. *Systematic method*

Description of the parcel. This should include the species of salmon, brand or label, packer, cannery, size of can, can mark, case mark, number of cases in the parcel, and location of the parcel.

Sampling. In canned salmon this is unsatisfactory at best. A parcel of 1,000 cases, containing 48,000 cans, may represent a cross section of the cannery's entire season's output ranging in extreme instances from very good to very bad. One case may even represent several days' canning. Under these conditions no systematic method of sampling can be carried out. Each case should be stamped with the date of packing, then if unsatisfactory fish are found a segregation can be made. All that can be done in sampling is to attempt to get a representative sample by taking one or two cans from a number of cases situated in all parts of the parcel. Ninety-six cans are usually drawn in this manner in parcels of 1,000 cases, and an increasingly smaller proportion from increasingly larger parcels. When the cases are opened for sampling they should be inspected for swollen or rusty cans and for thoroughness of lacquering and labeling.

Examination of the sample. This may be undertaken from five different viewpoints: (1). Bacteriological examination. (2). Workmanship in packing; including the vacuum, cleaning, filling, cooking,

salt, pugh marks and net weight. (3). Quality of the fish when caught; here considering the oil, amount of liquid, color and "fresh water marking" on the skin. (4). Condition of the fish when canned; involving odor, texture, reddening, "honeycombing" and turbidity of the liquid. (5). Chemical examination. These are considered in order below.

Bacteriological examination. From 24 to 48 cans are examined for living organisms. The cans are carefully cleaned and a gas flame is directed upon the top until all the moisture has been driven away and the lid thoroughly heated. The heating is not continued long enough to scorch the fish inside, however. After waiting a few minutes for the cans to cool, the vacuum is determined by means of a vacuum-pressure gauge. A hole is then made in the end near the seam by means of a hot, pointed, iron rod having a diameter of about 6 mm. ($\frac{1}{4}$ inch). By means of a sterile pipette, about 1 cc. of the liquid and finely divided particles of fish are transferred to a Petri dish and standard agar added. In a few cases 1 cc. of the liquid is also placed in anaerobic media. If any living organisms are found they are carefully examined to determine their nature. The presence of non-spore-formers indicates either a leaky can or contamination of the culture, the rigorous cooking to which salmon is subjected precluding the possibility of their survival. After the bacteriological sample has been taken the top of the can is cut off just below the seam and the liquid drained into a 15 cm. (6-inch) white enameled pan and poured into a graduated cylinder. The solid portion of the fish is then examined.

Vacuum. This is determined by means of a compound vacuum-pressure gauge equipped with a piercing point and a rubber gasket. A can should have sufficient vacuum, 8 inches (20.5 cm.) or more, to keep the ends concave under all conditions of temperature and altitude. Cans with tight seams will usually have some vacuum due to the absorption of oxygen by the salmon. Cans without vacuum will usually be found on close examination to have loosely rolled seams, a leak in the seam, or occasionally a small hole through the tin plate.

Cleaning. No pieces of the gills, fins or intestines should be found in the can. All clotted blood which could be removed without tearing the flesh should have been washed out.

Filling. There should not be more than three pieces of fish in the can and the long axis of these should parallel the long axis of the can. The ends of these pieces should be clean-cut, not jagged.

Cooking. This is judged as sufficient or insufficient according to the friability of the bone. If vertebrae are present these are pressed between the thumb and fingers to determine this point. In the absence of vertebrae the brittleness of the smaller bones is tested. Insufficient cooking as determined by this method does not necessarily mean that the cans are not sterile, nor does a soft, easily friable bone insure sterility. This test is much used in the salmon industry, however, and is a useful indication.

Salt. No definite amount can be stated since some people prefer more than others. However, there should be a definite salty taste.

Pugh marks. If fish are pughed before the blood in them has clotted, blood clots are formed along the puncture and may be found in the canned fish. They detract from the appearance of the pack and are an evidence of careless handling of the fish.

Net weight. The food and drug law of the United States, as amended, requires that every package of food be correctly marked with the net contents. Several cans are weighed and the average net weight calculated.

Oil. After the liquid has been standing in the graduated cylinder for a few minutes the oil rises to the top and the amount may be measured. When the fish begin their spawning migration they are usually fat, but since they no longer feed, this stored fat is gradually used up and the fish become poorer in quality. In deciding whether the oil in a can is good, average or poor, one must take into account the species, the section of the fish included in the can and the length of time the fish has been packed, since each of these factors has an effect on the amount of free oil in the can.

Amount of liquid. The total amount of liquid, including the oil, is measured and recorded. As the fat in a living fish decreases the amount of water increases; therefore, if a large amount of liquid is found in the can the fish is likely to be of inferior quality. The character of the liquid is recorded as normal, slightly turbid or turbid. The liquid in cans containing badly decomposed fish may be "milky" in appearance.

Color. Each species of salmon has its own characteristic color of the flesh, ranging from a deep red in the sockeye to a pure white in the white king. To a certain extent this color is modified in cooking, the degree of change varying with the different species. Furthermore, the amount of color within each species varies greatly with the

stage of the spawning migration at which they are caught, since the color gradually fades as one of the physiological changes of this period. During decomposition the natural color of the cooked fish also appears to fade gradually. The color of the cooked fish is expressed as good, average and poor, for that species.

"Fresh water marking" on the skin. The skin of a salmon which is on the spawning migration takes on various colors, some of them very bright. Jordan and Everman (1896) speak of these colors as the "nuptial dress," but in the trade they are known as "water marks," since they usually become noticeable after the fish have reached brackish or fresh water. Distinctly "water-marked" fish are nearly always inferior to those which do not have these colors.

Odor. This is one of the most reliable indications of decomposition and is usually the factor which decides whether a can of fish shall be condemned as unfit for food. In smelling the salmon it is first broken up between the hands and then held very close to the nose. The following terms, good, stale, tainted and putrid are the terms used in describing the odor. Fish which are canned while very fresh possess a normal fish odor. Fish canned when very slightly stale do not have this normal odor nor do they have a definite odor of decomposition. Both of these are recorded as good. Fish canned when stale will have abnormal odors, with a slight odor of decomposition, which usually leaves the fish during exposure to the air for a few minutes. These are recorded as stale, and are regarded as of poor quality but not unfit for food. Fish canned when tainted will have an unmistakable odor of decomposition, which persists in the fish even after exposure to the air for a few minutes. These are recorded as tainted and are regarded as unfit for food. When the odor of decomposition is very pronounced and offensive, apparent as soon as the can is opened, the odor is recorded as putrid. There are some odors which are encountered in canned salmon which, while abnormal, do not appear to be due to decomposition. Such an odor is found in "water marked" chum salmon.

Texture. Each species when packed fresh has its own degree of firmness. Fish which are stale or tainted before canning have a texture which is more or less soft, and the degree of softness corresponds roughly to the amount of decomposition in the fish, however this must be interpreted with care since this texture is also affected by the fatness of the fish.

Reddening. This is an important indication of spoilage. The flesh

of raw salmon takes on a feverish appearance, which persists through the processing; but when the can is opened the unnatural color will be found to be unevenly distributed and will fade quickly. It can thus be distinguished from the true color of the fish.

"Honeycombing." The canned product sometimes has small holes in the flakes which may extend entirely through them. It is probable that these holes are a result of the gaseous condition of the partially decomposed flesh before canning. When a piece of this "honeycombed" flesh is placed on the end of the tongue a distinct biting taste is experienced similar to the "bite" of strong cheese.

Turbidity of the liquid. The liquid in cans of salmon is, of course, always somewhat turbid, when the fish is stale or tainted before canning the turbidity is increased. Rough handling and very low temperature in the cans under examination may also result in a turbid liquid.

Chemical examination. Cans which have been classed as strongly stale by physical appearance and odor may be examined chemically for indol and skatol to determine whether they should be condemned as unfit for food. Cans which are good, slightly stale or tainted, as determined by odor, need not be examined chemically as their status is already well established by the preceding tests.

After carefully examining 48 cans (more for large parcels) according to the scheme outlined above, aided by an accurate record on forms similar to those in Fig. 1, the examiner is in possession of sufficient data to enable him to report on the thoroughness of the workmanship in packing the parcel, the quality of the fish when caught, their condition when canned and the adequacy of the cooking process.

3. CHEMICAL COMPOSITION OF FISH FLESH

One of the earliest investigators of the constituents of fish flesh was Morin, who in 1822 published his work on the composition of the smelt. Payen in 1854 determined the fat, protein, ash and water in the herring and the salmon. Several other chemists, among them Koenig (1876), Buckland (1874) and Almen (1887) also made similar analyses. The results obtained by them have been reviewed in detail by W. O. Atwater (1888) and recalculated to a common basis. The results which he and they obtained on salmon are given in table 3. The species is presumably the Atlantic salmon, *Salmo salar*.

TABLE 3. *Composition of the salmon.*

Year	Author	Water	Solids	Albuminoids	Fats	Ash	Protein N x 6.25
1854	Payen.	75.70	24.30	18.17	4.85	1.28	
1874	Buckland.	77.06	22.94	10.11	7.11	2.07	
1887	Almen.	70.33	29.67	18.06	10.12	1.49	19.39
1888	Atwater.	63.61	36.39	21.60	13.38	1.41	22.39

A considerable variation in composition will be noticed in table 3, which is probably due in large part to the seasonal changes in composition. Recent work by Clark and Almy (1918), Green (1919) and Dill (1921) has shown marked changes in several species of fish, especially at the spawning period. The principal variations are in the fat and water percentages which are inversely related to each other. The protein and ash percentages do not change to any great extent. A few examples are given in table 4.

TABLE 4. *Seasonal variation in the composition of fish.*

Author	Fish	Date	Water	Fat	Protein	Ash
Clark and Almy	Butterfish	May 19	74.34	5.96	18.06	1.49
Clark and Almy	Butterfish	Oct. 12	69.99	13.42	18.25	1.40
Dill	Yellow Fin Tuna	May 14	72.83	1.00	25.37	1.47
Dill	Yellow Fin Tuna	Sept. 8	69.17	6.54	24.00	1.32

Cobb (1921a) gives a number of analyses of Pacific Coast salmon made by Atwater (1888), Langworthy (1898), Knisely (1908), Loomis (1912) and Elliott and Clemens (1916). Most of these analyses represent canned salmon, but Loomis also analyzed a fresh sockeye salmon.

Recently Clark and Shostrom (Date?) have analyzed several hundred cans of salmon representing the five species of Pacific salmon from every important salmon canning district from northern California to the Yukon River in Alaska. The cans analyzed were all prepared from the second cut of an individual fish. This was done because the composition varies in different parts of the fish, and cans from the same part constitute a better comparison than those from different parts. Since this work was done in the National Cannery Laboratory at the University of Washington, Seattle, and has not yet been published, a summary of the results is given in table 5.

TABLE 5. *Composition of Pacific salmon.*

Species	No. individ- ual fish analyzed	Moisture %	Ether ⁴ extract %	Protein (N x 6.25) %	Total ash %	Salt- free ash %	Calories ⁵ per pound
Chinook (King)	204	63.53	13.50	19.48	2.85	1.18	931
Sockeye (Red)	130	64.52	10.84	20.67	2.97	1.29	841
Coho	99	66.26	9.47	20.40	3.15	1.22	778
Pink	90	69.24	6.16	20.56	3.47	1.32	642
Chum	120	68.95	7.42	20.83	2.40	1.24	700
Average, all	643	66.50	9.48	20.39	2.97	1.25	778

Fish flesh consists of protein, fat, water, mineral matter and a very small percent of carbohydrates. The composition of such fat fish as the Pacific salmon compares very well with the meat of mammals, but in general fish flesh is higher in water and lower in fat than other flesh and in consequence has a lower fuel value. However, eaten with carbohydrate foods, a balanced diet is obtained equal in fuel value and muscle building power to a similar diet containing other flesh. Recent unpublished work by Barton and McMillan (Date?) indicates the presence of a very small amount of carbohydrate material in salmon flesh.

The elementary composition of fish flesh was determined by Koenig and Splittgerber (1909) and compared with meat. Osborne and Heyl (1908) compared the hydrolytic products of halibut muscle with those of chicken muscle. Okuda (1919) has determined the cleavage products of both ordinary flesh and "chiai" flesh of the bonito, *Katsurwona pelamis* (table 6). The "chiai" flesh is the blood-colored flesh (dark meat) which occurs to a certain extent in the lateral muscle of most fish. The two kinds of flesh were separated as completely as possible and subjected to hydrolysis with mineral acid. Results are given in per cent of the ash and moisture-free muscle substance.

Okuda, Okimoto and Yada (1919) have published similar work on the whale and the cod; Okuda, Uematsu, Sakata and Fujikawa (1919), and Okuda (1919a), on the spiny lobster and the cuttlefish.

A great deal of disagreement occurs in the literature as to the amount of the different bases and amino-acids which are present in

⁴This represents fat.

⁵Calculated by the factors of Rubner: 18.6 Calories for 1 percent protein, and 42.2 Calories for 1 percent fat, on the basis of 1 pound (454g).

TABLE 6. *Products of hydrolysis of "chiai" and ordinary fish muscle.*

	"Chiai" muscle	Ordinary muscle
Alanin -----	1.1	2.3
Valine -----	1.8	2.8
Leucine -----	9.2	10.4
Proline -----	3.0	3.1
Phenylalanin -----	1.6	4.1
Aspartic acid -----	3.2	3.3
Glutaminic acid -----	12.1	8.1
Tyrosine -----	2.9	2.1
Arginine -----	7.08	7.8
Histidine -----	3.16	3.04
Lysine -----	6.78	7.41
Tryptophane -----	Present	Present
Glycocoll -----	Not found	Not found
Serine -----	?	?
Ammonia -----	0.78	0.64
Guanine -----	0.12	0.09
Adenine -----	0.1	0.04
Hypoxanthine -----	0.03	0.08
Xanthine -----	?	?
Creatine -----	0.29	0.44
Methylguanidine -----		0.005
Taurine -----	0.34	
Creatinine -----	Present	Present
Inosinic acid -----	.013	.043
Lactic acid -----	.067	.062

fish flesh. The methods used are rather difficult to carry out, and inexact, and it may also be that the amount in the flesh varies from time to time. The following results for creatin and creatinin in the flesh of the salmon will illustrate this.

Author	Creatin ⁶	Creatinin ⁶
Koenig and Splittgerber (1909) -----	0.027	0.207
Suzuki and Yoshimura (1909) -----	0.320	None
Okuda (1912) -----	1.525	0.182

Cholin, neurin and muscarin have not been isolated from fresh fish, but cholin was found by Bocklisch (1885) in herring brine, and by Mörner (1897) in "surfisk," a pickled, fermented fish product. Betaine has been found in the cuttlefish (Suzuki and Yoshimura 1909), the cod (Yoshimura and Kanai 1913) and the shark (Suwa 1909). Carnosin was found in the dried muscle of several fish, among

⁶Grams per 100 grams of dried fish.

them salmon (0.055%), by Suzuki and Yoshimura (1909). Urea has been found in large amounts in the muscle of several fish, notably the dogfish and skate (Benson 1920), while work in our laboratory indicates that the flesh of the barracuda, and the atka fish (from the Aleutian Islands) also contains urea. Taurin (Suzuki and Yoshimura 1909) and lactic acid (Liebig 1874) have also been found in small amounts, while according to Schondorff and Wachholder (1914) the glycogen content of fish muscle varies from 0 to 0.59%. The carp at death was found to have 0.527%, after one hour 0.359%, after one day 0.145%, and on the third day none. The muscle of fresh salmon and cod contained none. In fish livers the glycogen varied from 2.5 to 12.94%.

Oils are obtained both from the flesh of fish (fish oil) and from the liver (liver oil), but those fish which contain large amounts of fat in the liver have a small percentage of fat in the flesh. Little is known as to the composition of these oils. Stearic and palmitic acids, according to Lewkowitsch (1914), have been isolated from cod liver oil, but no oleic acid was found. Highly unsaturated acids are present but these are not identical with linolenic. Heyerdahl (quoted by Lewkowitsch 1914) concluded that the mixed fatty acids of cod liver oil contained about 4 per cent of palmitic acid, 20 per cent of jecoleic and 20 per cent of therapeutic acid. Liver oils contain cholesterol and other unsaponifiable matter. Fish oils contain palmitin. The mixed fatty acids contain highly unsaturated constituents which are not identical with linolic or linolenic acids. Tsujimoto (quoted by Lewkowitsch 1914) found clupanodonic acid to the extent of 6 to 9 per cent. Fahrion (1893) is of the opinion that he has proved the presence of jecoric acid in fish oil.

Beal and Brown (1921) have recently made a study of the fatty acids of five commercial fish oils, among them salmon oil. They found evidence of the presence of myristic, palmitic and clupanodonic acids and also for the presence of acids more highly unsaturated and of greater molecular weight than clupanodonic, such as hexadecatrienoic, arachidonic, eicosapentenoic, docosapentenoic, and docosahexenoic.

4. DECOMPOSITION OF FISH FLESH

When any organic material containing albuminous substances, such as fish, is allowed to stand under suitable conditions of moisture and of temperature, it decomposes very rapidly through the agency of enzymes secreted by various bacteria. This special fermentation,

known as putrefaction, differs from natural digestion in that the former yields many products not found in natural digestion, such as gaseous products, mercaptans, volatile acids, aromatic acids, amines, phenol, indol, skatol, and finally ptomaines. It was formerly thought that these products were excreted from the bacterial cells in which they had been formed, but it is now generally accepted that enzymes are secreted by bacteria, and these enzymes split the organic compounds, forming these and other new chemical substances.

Many different species of bacteria may be concerned in a spontaneous putrefaction. Bienstock (quoted by Rettger 1903, 1906) claimed that true putrefaction could be brought about only by obligate anaerobes. Rettger (1906) defined putrefaction as a bacterial decomposition of albuminous matter accompanied by the formation of "Faulnisprodukte." In his earlier work, Rettger (1903) took exception to Bienstock's conclusions, but in later work (1908), using extreme care, his results confirmed those of Bienstock. Effront (1917) states that, "The putrefactive bacteria are ordinarily anaerobic, like *Bacillus putrificus coli*; nevertheless there are also very active ones which are aerobic, like *Bacillus coli communis*." Moreover, the bacterial flora is not the same throughout the putrefaction, some species converting the original proteins into substances which other bacteria can utilize, and frequently forming substances which are injurious to themselves. According to Effront, none of the putrefactive bacteria produce pepsin, but several produce trypsin which converts the albuminoid material into albuminoses, peptones and amino-acids, which are then attacked by erepsin, secreted by other species, and changed into simpler compounds. Finally, amidases come into action and bring about the formation of volatile acids and amines, as well as phenol and indol derivatives.

The following substances are given by Effront as likely to occur in the course of putrefaction in addition to gaseous products (CO_2 , CH_4 , N_2 , H_2S , PH_3) and residual peptones: "(1) Ammonia and amines; ethylamine, propylamine, and trimethylamine. (2) Volatile acids, comprising all the members of the fatty series up to caproic acid. They are sometimes normal acids, sometimes their isomers; propionic acid is less frequent than the others; formic acid is quite rare; acetic and butyric acid are especially common. (3) Aromatic acids and oxyacids; like phenylpropionic, oxyphenylacetic, and oxyphenylpropionic acids. (4) Phenol, indol, scatol, pyrrol and its derivatives, these bodies sometimes being in very small quantities, or even completely absent. (5) Sulphur derivatives, like methyl-mercaptan. (6) Various amino-acids; leucin, tyrosin, tryptophane, and

sometimes glycin, creatinin, etc. (7) Various ptomaines; like putrescin and cadaverin, the guanidins, cholin and neurin, pyridin, hydrocollidin, etc."

In addition to the products formed by the splitting of nitrogenous compounds, there will also be decomposition products of the fats, consisting of glycerin and various fatty acids. The following reactions show how some of the above substances may be formed during decomposition.

The effect of amidases on amino-acids was studied by Effront (1911), who gives the following reactions: (1). The monobasic acids are transformed into ammonium salts, e.g., glycin with the addition of hydrogen gives ammonium acetate. (2). Betain by the addition of hydrogen and the loss of water is transformed into the acetate of trimethylamine. (3). The polybasic acids undergo a molecular degradation, e.g., aspartic acid by the addition of hydrogen yields ammonium propionate and carbon dioxide.

Tanner (1917) gives three methods by which straight chain acids may be simplified: (1). Deaminization; glycin to acetic acid and ammonia. (2). Decarboxylation; glycin to methylamin and carbon dioxide. (3). Oxidation; acetic acid to carbon dioxide and water. Tanner also shows by reactions the probable steps in the formation of phenol from tyrosin: (a) Tyrosin; (b) parahydroxyphenylpropionic acid; (c) hydroxyphenylacetic acid; (d) paracresol; (e) phenol.

Ehrlich (1909) has shown that amino acids may be fermented into alcohols corresponding to the acids used, ammonium bicarbonate being formed simultaneously. The acids which are thus formed may be very quickly changed to simpler ones; glutamic acid, which probably first forms oxybutyric acid, yields succinic acid as a final product.

Hopkins and Cole (1903) give the following steps in the decomposition of tryptophan by bacteria: (1) Tryptophan; (2) Indolpropionic acid; (3) Indolacetic acid; (4) Skatol; (5) Indol. The decomposition outlined above is the probable source of the indol found in our raw and canned salmon experiments.

Effront (1917) says that tyrosin by the addition of hydrogen may yield methane and ammonium-p-oxyphenylacetate, a reaction which may explain the formation of methane from albuminoid material.

Ornithin may give rise to either amino-valeric acid or to tetramethylenediamine; in the first case by the addition of hydrogen and in the second by the loss of carbon dioxide. Lysin may be transformed to pentamethylenediamine and carbon dioxide. Another ptomaine,

neurin, is derived by loss of water from cholin, which is the constituent base of the lecithins.

According to Mathews (quoted by Tanner, 1919) cystine goes to cysteine, then to thioethylamin and finally to ethyl mercaptan. Tanner (1917) has shown that bacteria can produce hydrogen sulphide from cystine.

Effront (1917) speaking of the gaseous products of putrefaction states that the digestive enzymes (pepsin, trypsin, and erepsin) do not have an appreciable effect on the sulphur contained in albuminoid material, but that putrefaction enzymes split it off in the form of H_2S and the mercaptans. Methane can arise from the reduction of tyrosin and carbonic acid as a by-product in many of the reactions already given. Ammonia is also a by-product of many reactions. However, as to the mechanism by which nitrogen, hydrogen and hydrogen phosphide are formed, nothing is known. He is inclined to attribute the formation of nitrogen to the reduction of nitrates rather than to albuminoid material.

Effront points out that substances formed early in putrefaction may not be present at later stages. Some of the poisonous substances elaborated during decomposition may disappear later, and it is more dangerous to eat meat just beginning to putrefy than that which is completely decomposed. Duclaux states that phenol and indol may be formed and then decomposed during the course of putrefaction; but Effront states flatly that "when we do not find indol present, it is because it was never formed."

The reactions and data given above show that it is impossible to give a simple and unchangeable scheme for decompositions since "the quality and quantity of the products formed are dependent upon the nature of the acting enzymes, which, themselves, are functions of the species of bacteria present, of the albuminoid substance to be transformed, and also of the physical and chemical conditions of the medium."

Fish flesh, as has been shown, contains most of the amino-acids and other nitrogenous substances just mentioned, and presumably all of the decomposition products listed may be formed from them under suitable conditions. The question arises as to which one of these products can be used to the best advantage as an index of the amount of decomposition in a product such as canned salmon. Ammonia has been frequently used as a measure of decomposition and seems to be fairly satisfactory when dealing with raw materials; but when used with canned products, such as meat and fish, the results show a decidedly disturbing factor, i.e., the ammonia produced by the cooking

process during canning. On account of this factor the investigator of canned meats and fish is left in doubt as to the percentage of the ammonia present in canned goods which is due to the canning process, and the percentage which is due to decomposition before canning. Loomis (1912) determined the "ammoniacal nitrogen" in fresh and canned salmon by two methods and makes the following observation. "As all samples of canned salmon were in good condition and gave no indication of deterioration as far as the senses could detect it, the results on 'ammoniacal nitrogen' are also of interest, being two or more times greater in the case of the canned product than in the fresh fish."

Weber (1921) made an experimental pack of sardines in different stages of spoilage and determined the volatile nitrogen as ammonia and amines both before and after canning. He concludes that, "The cooking received during sterilizing very greatly increased the amount of ammoniacal material in the packed fish." He further states that in the case of fish which had undergone an excessive decomposition his results indicate that the determination of volatile alkaline material may be used to detect this degree of spoilage but that with lesser amounts of decomposition the method is of doubtful value.

Bidault and Couturier (1920) state that the quantity of the ammoniacal compounds in canned meat is a function of the temperature of sterilization.

The amount of free fatty acid undoubtedly increases during decomposition. Weber made a large number of determinations in his work on the **sardine**, but from the fact that only a few of the results are given in his report, and that these are very inconclusive, we may conclude that little reliance may be placed on the method. No reference was found in the literature to the effect of the canning process on the free fatty acids of canned foods; however, since one of the principal methods of hydrolysis of fats consists in heating the fats under pressure in the presence of steam, it seems highly probable that some of the fat in salmon may be hydrolyzed during the canning process, since all the factors of steam, heat and pressure are present.

Röhmman (1908) states that tryptophan, when heated, gives rise to indol and skatol. Salmon flesh contains tryptophan, and the question arises as to whether the canning process is severe enough to break it down to indol or skatol. Experiments herein recorded prove that no considerable amount of these compounds is formed during the ordinary canning process. In fact, the canning process, as shown

by further experiments, does not appear either to increase or decrease the amount of these compounds already present in partially spoiled salmon. No references were found in the literature as to whether indol or skatol had been found in canned salmon, but work done by Houghton and Hunter (1920) of the U. S. Bureau of Chemistry, published several months after this investigation was initiated, showed that it was frequently present and might possibly furnish a means of detecting spoilage.

From a consideration of the above information relative to ammonia, fatty acids, and indol and skatol, it appeared that the latter were the most promising decomposition products to use as an index of spoilage. It was therefore decided to investigate the presence of indol and skatol in raw and canned salmon, and to attempt to develop a quantitative method of determination which should be available as a check on the organoleptic examination of the latter. Some work was also carried out, using ammonia and fatty acids as indexes of decomposition.

5. DEVELOPMENT OF A METHOD FOR DETECTING DECOMPOSITION BY MEANS OF INDOL AND SKATOL

A. *Selection of suitable color tests*

From a study of the literature relating to the decomposition of nitrogenous matter it seemed that the formation of indol and skatol would form the most accurate index of the presence and progress of decomposition. The next step therefore was to proceed to select a method for the determination of these substances. Owing to the small amount present even in very advanced stages of decomposition, this method must necessarily be based on a color reaction. Fortunately both indol and skatol give very marked colors in extremely minute quantities. Numerous color reactions are recorded in the literature, and among them are the following.

Indol

a. *Formaldehyde reaction* (Konto 1906). To 1 cc distillate in a test tube add 3 drops of a 4% formaldehyde solution and 1 cc. of concentrated sulphuric acid. Agitate the mixture and observe the appearance of a violet-red color if indol is present. Konto states that indol may be detected in a dilution of 1:600,000. Skatol gives a yellow or brown color.

b. *Cholera-red reaction* (Salkowski, 1883; Tobey 1906a; Hawk 1918). To 5 cc of the distillate in a test tube add one-tenth its volume of a 0.02 per cent solution of potassium nitrite and mix thoroughly. Carefully run concentrated sulphuric acid down the side of the tube so that it forms a layer at the bottom. Note the purple color. Neutralize with potassium hydroxide and observe the production of a bluish-green color.

c. *Nitroprusside reaction* (Deniges 1908; Hawk 1918). To a small amount of the material under examination in a test tube add a few drops of a freshly prepared solution of sodium nitroprusside, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} + 2\text{H}_2\text{O}$. Render alkaline with potassium hydroxide and note the production of a violet color. If the solution is now acidified with glacial acetic acid the violet is transformed into a blue.

d. *Nitroso-indol nitrate test* (Hawk 1918). Acidify some of the material under examination with nitric acid, add a few drops of a potassium nitrite solution and note the production of a red precipitate of nitroso-indol nitrate. If the material contains but little indol simply a red coloration will result.

e. *Vanillin-sulphuric acid test* (Steensma 1906; Deniges 1908; Blumenthal 1909; Nelson 1916; Zoller 1920; Weehuizen, (date?). To 5 cc of the solution add 5 drops of 5% solution of vanillin in 95% alcohol, 2.5 cc of concentrated sulphuric acid and mix. If indol is present an orange color will be formed. Test is sensitive to 1 part in 2 million. If skatol is present a violet color will be formed. Test is sensitive to 1 part in 4 million.

f. *Para-dimethylaminobenzaldehyde* (Herter 1905; Steensma 1906; Deniges 1908a; Von Moraczewski 1908; Blumenthal 1909; Baudisch 1915; Nelson 1916; Ingvaldsen and Bauman 1920; Zoller 1920). To 5 cc of solution, add 2 cc of a 2% alcoholic solution of paradimethylaminobenzaldehyde, 10 drops of concentrated hydrochloric acid and mix. After a few minutes, add 1 cc of chloroform, shake and allow chloroform layer to separate. If indol is present a purplish red color is formed. Test is sensitive to 1 part in 1,000,000. Skatol produces a faint bluish color in dilutions of 1 part in 100,000.

g. *Beta-naphthaquinone reaction* (Herter 1905; Herter and Foster 1905, 1906; Bergheim 1917; Hawk 1918; Zoller 1920). To a dilute aqueous solution of indol (1:500,000) add 1 drop of a 2 per cent solution of B-naphthaquinone-sodium-mono-sulphonate. No re-

action occurs. Add a drop of a 10 per cent solution of potassium hydroxide and note the gradual development of a blue or blue-green color which fades to green if an excess of the alkali is added. Render the green or blue-green solution acid and note the appearance of a pink color. Heat facilitates the development of the color reaction. One part of indol in 1,000,000 parts of water may be detected by means of this test if carefully performed.

h. *Pine wood test* (Hawk 1918). Moisten a pine splinter with concentrated hydrochloric acid and insert it into the material under examination. The wood assumes a cherry-red color.

i. *Oxalic acid* (Morelli 1908). Oxalic acid either solid or in concentrated solution takes on a red color with indol or indol vapor. Blotting paper soaked with oxalic acid solution introduced into the incubator or hung over a culture dry or moist, reacts very sensitively for indol produced by the bacteria.

j. *Furfural* (Escallon and Sicre 1906). Extract culture with chloroform; drive off chloroform from the extract; take up residue in a few drops of alcohol, warm with 3 cc of the furfural reagent (glucose 1 gram and HCl 5 cc, warm to boiling, make up to 100 cc with water). Indol gives a reddish orange color.

k. *Glyoxylic acid* (Dakin 1906). To 1 cc of solution to be tested add 1 cc of a solution of calcium glyoxylate (containing 0.1 mg per cc) and 2 to 2.5 cc pure sulphuric acid. Note color at zone of contact and then slowly mix. Red color. Indol may be detected in a dilution of 1:200,000 and skatol 1:1,000,000.

l. *Pyruvic aldehyde* (Nelson 1916). To 5 cc of the solution to be tested add a small crystal of ferric sulphate and a few crystals of pyruvic aldehyde. A layer of concentrated sulphuric acid is then added and if indol is present a violet ring is formed. Indol may be detected in a dilution of 1:500,000.

Skatol

a. *Dimethylaniline test* (Nelson 1916). To 5 cc of the solution to be tested add a few drops of dimethylaniline and shake vigorously. Add about 4 cc concentrated sulphuric acid to form a layer at the bottom. Violet ring is formed in dilutions of 1:1,000,000 or more. Color soluble in chloroform. Indol does not interfere.

b. *Para-dimethylaminobenzaldehyde reaction* (Hawk 1918). To

5 cc of the distillate or aqueous solution under examination add 1 cc of an acid solution of para-dimethylaminobenzaldehyde (made by dissolving 5 grams of para-dimethylaminobenzaldehyde in 100 cc of 10 per cent sulphuric acid) and heat the mixture to boiling. A purplish-blue coloration is produced which may be intensified through the addition of a few drops of concentrated hydrochloric acid. If the solution be cooled under running water it loses its purplish tinge of color and becomes a definite blue.

c. *Glyceric aldehyde* (Nelson 1916). To the solution to be tested add a drop or two of glyceric aldehyde and sulphuric acid. Skatol produces an intense red color, soluble in chloroform, while indol gives a yellow color insoluble in chloroform.

d. *Methyl alcohol* (Sasaki, date ?). To the solution to be tested add 3 or 4 drops of methyl alcohol and an equal volume of sulphuric acid. The acid must contain a trace of a ferric salt and the alcohol must be free from acetone. Reddish violet color produced with skatol. Indol does not interfere.

e. *Para-dimethylaminobenzaldehyde* (Blumenthal 1909; Steensma 1906). Test as for indol. Reaction not as delicate.

All of the methods given above except *i*, *j*, *k* and *l* for indol, and *c* for skatol, were tried experimentally; from them Ehrlich's test for indol (*f*) and either Herter's test (*b*) or the dimethylaniline test (*a*) for skatol, were selected as the most suitable for our use. Tests for indol, except *e*, *f* and *g*, and tests for skatol, except *a*, *b* and *e*, are not sensitive enough to be used with amounts of 1mmg indol or skatol.

The vanillin test for indol (*e*) is extremely delicate but frequently gives abnormal colors. Many substances other than indol give a similar color with vanillin. Experiments showed that HCl might be substituted for H_2SO_4 ; the test was fully as sensitive and the charring effect of the concentrated H_2SO_4 on organic substances was avoided. This test was frequently used as a confirmation test. A number of substituted vanillins were tried as color reagents and compared with vanillin. They proved to be less sensitive than vanillin and were discarded.

B. *Modification of the selected color tests*

Ehrlich test for indol (*f*) being chosen as the one most suitable for our work on raw and canned salmon, experiments were under-

taken to increase its delicacy. The amount of indol in stale salmon is very small and the test to be used for its detection and estimation must be extremely delicate. The experiments were along the following lines:

The color of the glass of the test-tubes needs to be considered. It was frequently found that amounts of indol which should have given a definite color apparently gave little or none. On investigation it was found that some of the test tubes in use were made of glass which had a decided bluish-green color which served to mask partly or completely the faint pink color produced by small amounts of indol by either the Ehrlich or Vanillin test. Since we were attempting to record amounts of indol as small as 0.2-0.3 mmg the test tube was a big factor. Only thin test tubes of nearly colorless glass and as nearly as possible of uniform size were used in the experiments on raw and canned salmon.

P-dimethylaminobenzaldehyde dissolved in alcohol has a strong yellow color which tends to mask the pink color in faint indol tests. By reducing the amount of reagent this interference is partially eliminated. Half a cc of the Ehrlich reagent was the amount selected as the best for small amounts of indol (2.0 mmg or less).

Concentrated HCl partially destroys the yellow color of the p-dimethylaminobenzaldehyde reagent which interferes with the pink indol color. Since the 10 drops usually used have only a small effect, larger amounts were tried. A part of the yellow color was destroyed and the pink color became much more prominent. The indol color must be estimated at once however since it also is destroyed by the acid on standing. One cc was the amount selected as best for use with small amounts of indol.

The influence of heat on the color was observed. After several experiments with the indol test, it was found that the color developed much faster when the test tubes were heated than when left at room temperature. However the time of heating had to be short or the color was partially destroyed by the acid. Heating for 20 seconds appeared to be the most favorable treatment.

A modified Ehrlich method is given here. *Reagents:* (a) Para-dimethylaminobenzaldehyde, 2 grams in 100 cc of 95% alcohol; (b) HCl, 600 cc concentrated plus 200 cc of water; (c) Chloroform, U.S.P. *Method:* To 5 cc of the water test solution add 0.5 cc reagent *a* and 1 cc reagent *b*. Place in boiling water bath for about 20 seconds, shaking vigorously, then place in ice water about one-half minute and extract with 1 cc reagent *c*. Comparison is

made with standards prepared in exactly the same way. *Delicacy*: With pure water solutions of indol, 0.2 mmg may be easily detected in 5 cc, a dilution of 1:25,000,000.

Herter's test for skatol (b) is a very delicate one for both skatol and indol, but as in the Ehrlich test, the yellow color of the p-dimethylaminobenzaldehyde tends to obstruct the faint pink or blue produced by minute quantities of indol or skatol. Experiments indicated that 0.5 cc of the reagent to 5 cc of the solution to be tested gave better results than 1 cc. With this amount of reagent a distinct pink or blue could be obtained with 0.5 mmg indol or skatol in 5 cc of test solution, a dilution of 1:10,000,000. The distinction between indol and skatol is very marked even at this concentration, whereas the colors produced by the vanillin test on dilute solutions are very much alike. Substitution of 10 per cent HCl for 10 per cent H₂SO₄ in making up the reagent was found advantageous, since the sulphuric acid tended to char organic substances in the test solutions obtained from fish, obscuring the results of the test.

A modified Herter's method is given here. *Reagents*: (a) Para-dimethylaminobenzaldehyde, 5 grams in 100 cc of 10% HCl; (b) Concentrated HCl; (c) Chloroform, U.S.P. *Method*: To 5 cc of the water test solution add 0.5 cc of reagent *a* and heat nearly to boiling. Add a few drops of reagent *b*, cool, add 1 cc reagent *c* and shake vigorously. Comparison is made with standards prepared in exactly the same way. *Delicacy*: Either indol or skatol could be easily detected, when present to the extent of 0.5 mmg in 5 cc water, a dilution of 1:10,000,000.

The dimethylaniline test for skatol (a), as given in some unpublished work by the U.S. Bureau of Chemistry, prescribes sulphuric acid. Experiments showed that hydrochloric acid might be used instead and the charring effect of the sulphuric acid avoided. In this test heat should be used to bring out the color. Skatol gives a pink color; indol does not interfere unless present in very large amount.

A modified dimethylaniline method is given here. *Reagents*: (a) Dimethylaniline, C.P. and recently redistilled; (b) Concentrated HCl. *Method*: To 5 cc of the solution to be tested add 5 drops of reagent *a* and shake vigorously. Add 4 cc reagent *b* and heat in a water bath. *Delicacy*: With pure water solutions of skatol, 1.0 mmg may be easily detected in 5 cc, a dilution of 1:5,000,000.

C. Distillation of indol from salmon

Indol and skatol, as well as some of the other products of decom-

position, are volatile with steam; and the question as to how completely they may be separated from such a material as fish by steam distillation at once arises. Zoller (1920a) has determined the percentage of recovery of indol from culture solutions of different pH concentrations, and concludes that a slightly alkaline solution (pH 9) gives the highest percentage. Bigelow and Cathcart (1921) give the pH value of canned salmon as 6.25, and the five species apparently do not differ appreciably. Furthermore, salmon in various stages of decomposition have been found to differ very little in hydrogen ion concentration. In order to ascertain the effect of reaction on the amount of indol recovered we made a number of distillations of water solutions of indol, using our regular method, with the exception that varying amounts of alkali or acid were added to the flask. The same amount of indol (12mmg) was used in each distillation, and 500 cc was distilled. The recovery in none of these cases was very satisfactory, but nearly twice as much was recovered from the alkaline solutions as from the acid.

Zoller also states that a higher percentage of recovery is attained when the volume of the liquid in the distilling flask is reduced as far as possible. We made a number of distillations, in some of which the liquid in the distilling flasks was allowed to increase, and in some the volume was much decreased. Twelve mmg indol were used and 500 cc distilled in each distillation. Reducing the volume increased the percentage of recovery.

The effect of adding NaOH to the fish in the distilling flask was next tried, but so much difficulty was experienced from frothing by the alkaline mixture, that this treatment was not considered feasible. Furthermore, Bigelow's work showed that canned salmon was nearly neutral, and correction of reaction was not as necessary as with more acid substances.

The percentage recovery of added indol from flasks containing fish formed the subject of the next experiments. Some fresh salmon was obtained from the market, skinned, boned and ground very fine, becoming thoroughly mixed in the process. A blank run on this salmon indicated that it was free from indol. Varying amounts of this fish with varying amounts of water and added indol were distilled and the percentage of recovery determined. The results showed that the recovery from fish was fully as good as that from water alone. They also showed that most of the indol which can be recovered from amounts of the magnitude used in these experiments is recovered in the first 500 cc of distillate.

To determine at what stage of the distillation most of the indol formed in decomposed salmon comes over, a series of experiments, using fish in various stages of decomposition, was carried out. These fish had been packed after being out of water from one to six days, and an idea of their condition can be obtained by consulting the data on chum salmon (6, F, below). Two hundred grams of fish, with 200 cc of water, was placed in the flask and distilled at a uniform rate. The distillate was collected in 100 cc amounts and the indol in each determined as carefully as possible. The liquid in the flask (water test solution), after the evaporation of ether, frequently required dilution, in order that the color produced might not be too intense.

The results of these experiments showed that when small amounts of indol are present a very large percentage of all that it is possible to distill from the fish or other material is obtained in the first 500 cc. With larger amounts the percentage grows smaller until, when using fish canned when 6 days old, the amount recovered in successive 100 cc portions was not strikingly less in distillate 7 than in distillate 1. The fact that the percentage obtained in the first 500 cc was rather small in the case of large amounts of indol was not particularly disturbing, since it was proposed to use the method as a measure of decomposition only in those cases in which the sense of smell was in doubt, in which cases only small amounts of indol, up to 6 mmg or less, were likely to be found. Therefore, in working with salmon it appears that it is satisfactory to distill one portion of 500 cc without the addition of alkali. An effort should be made to decrease the volume of liquid in the distilling flask to as small an amount as will still leave the contents in a fluid condition.

D. *Extraction of indol from the distillate*

The 500 cc distillate is transferred from the 600 cc beaker to a liter separatory funnel and extracted once with 100 cc of ether. Both ethyl ether and petroleum ether were used and the former finally selected as the most suitable, for several reasons. It was more easily obtained than petroleum ether of good grade. Frequently the latter could not be obtained from the local supply house and it was necessary to distill ordinary gasoline and use the low-boiling fraction. Frequently petroleum ether bought as low-boiling (below 60° C.) proved to contain 40% or more boiling above that temperature. Furthermore, some samples of petroleum ether contained impurities which interfered with color reactions, particularly with the vanillin

sulphuric acid reaction. Nearly every can of U.S.P. ethyl ether proved to be free from interfering substances and of course boiled at a constant low temperature. It was therefore used exclusively in the raw and canned salmon experiments.

When the distillate was violently shaken in the separatory funnel with the ethyl ether, a persistent emulsion usually resulted, from which only a part of the ether could be separated and also only a part of the indol. A quantity of concentrated HCl (10 cc) was therefore added to the funnel before shaking, which prevented the formation of an emulsion and gave a sharp separation of the ether and water. Care should be taken to use c.p. HCl, since there appears to be some substance in commercial HCl which interferes with the color test. Although only about 50 per cent of the ether used in the first extraction separates from the water, practically all of the indol which can be recovered is secured in the first extraction.

During decomposition other products than indol and skatol are formed, and since some of these may distill with steam and interfere with the color reactions for indol and skatol, the ether extracts of the distillate are washed with dilute NaOH (2.5%). Repeated experiments showed that washing with alkali alone rendered the water test solution alkaline and also interfered with the color reaction; however, if the ether was rewashed with dilute acid (10 cc concentrated c.p. HCl in 200 cc water) no interference with the color reaction was experienced. Repeated experiments showed that washed ether extracts gave clearer and slightly more intense color tests than exactly similar unwashed ether extracts.

E. *Evaporation of the ether extract*

After the ether extractions have been washed with alkali and acid they must be evaporated over a small amount of water (the test solution). This evaporation may be allowed to take place spontaneously; or be hastened by a current of air drawing away the ether vapor; or by heating, either on a hot plate or a water bath, or by immersion of the flask in hot water. All of these methods were tried in order to find which gave the least loss of indol. In each method the same quantity of ether (130 cc) received different amounts of indol in 15 cc of water and after evaporation the water was divided into three equal portions and the indol determined in each by a different method. The results indicated that some method of evaporation by heat was preferable to spontaneous evaporation, and among

the heating methods that of the steam bath seemed to be the best by a slight margin.

TABLE 7. *Loss of indol during the evaporation of the ether extract.*

Method	Indol added mmg.	Indol recovered in mmg.			
		Ehrlich's test	Herter's test	Vanillin test	Total
Spontaneous evaporation	12.	2.2	1.8	3.0	7.0
Immersion in hot water	12.	3.0	1.8	2.8	7.6
Hot plate	12.	3.0	3.0	3.8	9.8
Water bath and aspirator	12.	3.8	2.8	4.0	10.6

The temperature of the water test solution under the ether remains at about 40° C. until nearly all of the ether is gone, when it rapidly rises, and great care must be exercised at this point to take the flasks off the water bath while there is still a little ether left. The last traces of ether may be removed by rotating the warm flask and drawing air through it by an aspirator. All the ether must be removed before the test solution is divided for the color tests.

The amount of water used for the test solution varies but is usually 10 cc. This amount enables one to make check color tests on two 5 cc portions; or if the first 5 cc portion gives a color too intense for comparison with the standard colors, the second 5 cc may be diluted to any desired extent in order to reduce the amount of indol in 5 cc to about 3 or 4 mmg, which amounts give colors of maximum ease of comparison. Experiments were made which proved that such dilutions could be safely and accurately made.

F. *Color tests on the water test solution*

After the ether has been entirely removed from the flasks containing the test solutions, these are ready to be subdivided and the color tests made.

Usually the water test solutions consisted of 10 cc which was equally divided in two thin-walled, colorless, glass test tubes, one used for the test and one held in reserve. The tubes were placed in a rack in numerical order; and a series of standard tubes containing 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0 and 6.0 mmg indol in 5 cc of water arranged in another rack. A series of reagent burettes arranged on a revolving stand contained the following reagents: Water, standard indol solution, p-dimethylaminobenzaldehyde solution, hydrochloric acid, chloroform, and sometimes a modified Herter's reagent and a standard skatol solution. A boiling water

bath, having a basket of coarse wire screen inside to keep the test tubes from falling, and two beakers of ice water, completed the arrangements. Each tube received the reagents, was heated 20 seconds in the boiling water, and was plunged into ice water to cool, while a second tube received reagents and heating; after which the first tube was moved to the second beaker, and a third tube received reagents and heating. As soon as this tube was ready for the ice water, the first tube was cool enough to be extracted with 1 cc of chloroform, when it was placed in its proper place in the rack. By following this routine, each tube, in both the standard and unknown solutions, received exactly the same treatment. As soon as all the tubes had been treated they were compared with the standard tubes by holding in front of a piece of white paper and the color in the chloroform estimated as accurately as possible. The results were recorded in terms of mmg, on a basis of 100 grams of fish used.

Standard solutions of indol and skatol contained 4 mg per liter, and they were made up fresh about every day, for experiments proved that they lost strength very rapidly. Owing to the difficulty in weighing accurately so small an amount of indol as 4 mg, alcoholic solutions containing 80 mg in 100 cc were prepared. By pipetting 5 cc of the strong solution into a liter volumetric flask and filling to mark, a standard solution was easily and accurately prepared. Such concentrated solutions in alcohol, if tightly stoppered, will keep for months.

G. *The method as finally developed*

The substance to be examined is thoroughly mixed, by grinding in a meat grinder, if necessary, as in the case of raw salmon, and a sample weighing 200 grams transferred to a liter, round bottomed, long necked flask, using about 200 cc of water. A current of live steam is then passed through the mixture in the flask until 500 cc of distillate are collected. A gallon oil can, having a long glass safety tube placed in the opening on top and a rubber tube attached to the spout, makes a very satisfactory steam generator. The steam is passed through the fish by means of a glass tube reaching to the bottom of the flask (flask inclined at an angle of about 45°) and so bent near the end as to give a rotary motion to the contents as the steam issues from it. The flask is kept boiling hot by being placed in a boiling, saturated salt solution. The steam from the flask is received in a vertical worm condenser, the end of which projects below the surface of a small amount of water in the receiver (600 cc

beaker). The distillate is transferred to a liter separatory funnel, acidified with 10 cc c.p. concentrated HCl and extracted with 120 cc ethyl ether (U.S.P.) with repeated and vigorous shaking of the funnel. After the ether has separated, the ether layer is transferred to a 250 cc separatory funnel and washed first with 25 cc of NaOH solution (2.5%) and then with 25 cc dilute HCl (10 cc c.p. concentrated HCl plus 200 cc water). The first washing is to remove compounds which might interfere in the color tests, and the second to neutralize any alkali left in the ether. The ether is then placed in a small flask with 10 cc of distilled water and evaporated on a steam bath, taking great care that while the last of the ether is being driven off the water layer is not heated appreciably above the boiling point of ether, since the indol may be easily lost by volatilization at this stage. A 5 cc portion of the 10 cc water residue is now tested for indol, and 5 cc for skatol, by the modified tests before described (5, B).

6. EXPERIMENTAL WORK ON THE FIVE SPECIES OF SALMON IN DIFFERENT STAGES OF DECOMPOSITION

A. *General outline of the method employed*

Having now developed a satisfactory method for the determination of indol and skatol in salmon, a comprehensive study of raw and of canned salmon was planned for the purpose of determining the significance of the presence of these decomposition products. This study was to cover the physical changes, the appearance and increase of indol or skatol, and a qualitative and quantitative investigation of the bacterial flora during progressive decomposition, together with such correlations among the physical, chemical and bacteriological changes as could be discovered.

These experiments will first be described in general, and any divergence from the general method will be given in the portion devoted to each species of salmon. In these portions the physical, chemical and bacteriological condition of each species of fish at the time of inspection are given in daily average tables showing the progressive nature of decomposition under each factor studied and in charts giving the results of the indol tests in both raw and canned salmon.

Dr. Carl R. Fellers, the bacteriologist of the laboratory, took charge of the bacteriological work, while Mr. Oscar E. Shostrom, assistant chemist, and I, were responsible for the physical and

chemical problems. Doctor Fellers has very kindly permitted me to include a part of the bacteriological results, in order to show the correlations between bacterial flora and indol and skatol content.

The salmon used in these experiments were obtained as they were taken from the traps, in order that there might be no question as to their exact age out of water. They were placed in boxes holding about 10 or 12 salmon each, and stored on the dock over the water, under cannery conditions as nearly as possible. In every case the traps were lifted early in the forenoon. The next morning and each succeeding morning (even periods of 24 hours each) one-sixth of the fish were brought from the dock to the laboratory by automobile. A maximum-minimum thermometer was placed with the fish stored on the dock, and each morning the range of temperature for the preceding 24 hours recorded. The fish taken each morning were selected without regard to their condition, some from the top and some from the bottom of the boxes. Immediately upon arrival at the laboratory, each fish received a designating letter, was hung upon a board which had been painted white and divided into six-inch (153-millimeter) squares with black lines, and was photographed with an ordinary kodak having a portrait lens attached. The raw salmon were then examined as follows⁷:

Physical examination. The salmon were laid in order on a large table, measured and weighed. Three of each species were again weighed after cleaning to show the percentage loss. The general appearance of the fish was then recorded as indicated below.

The skin, as the fish is taken from the water, is bright and free from slime, but it gradually grows dull and much slime appears. This slime forms an excellent medium for the growth of bacteria and helps to distribute them from fish to fish.

The scales adhere firmly at first but gradually grow looser.

The gills are bright red at first, gradually growing gray and finally greenish gray. Frequently the gills on one side of a fish were red while those on the other side were gray.

The eyes are bright, transparent and slightly bulging, when the fish is taken from the water. They gradually become bloodshot or gray, and less prominent, finally becoming sunken; in extreme cases only the socket may remain.

⁷In the original typewritten thesis, deposited in the library of the University of Washington, a separate page is devoted to each of the 138 fish examined, giving all the physical, chemical and bacteriological data obtained, together with a photograph of the fish. Owing to the expense of printing, these data on individual fish have been omitted. However, the data have been averaged by days and are presented in tables, thus showing the decomposition changes from day to day.

The elasticity changes. Shortly after death the fish becomes rigid in *rigor mortis* and the flesh is firm. This rigidity is lost after a few hours but the flesh remains firm for some time. The flesh is elastic; where pressed in it immediately springs out again as soon as the pressure is removed. This response becomes slower and slower until finally impressions made with the thumb or finger remain.

Fly-blows and maggots are sought. Flies soon gather about a pile of fish; unless the heaps are well protected they receive masses of eggs. In about 24 hours these produce maggots, resulting in a very filthy condition.

Bacteriological examination. The scales were scraped from a small area near the back, close to the dorsal fin, and a hot iron was pressed against the skin until it was thoroughly seared over an area of about three square inches (58.5 sq. cm). With sterile scalpels and needles an incision was made to a depth of an inch (2.5 cm) or slightly less, and a piece of flesh weighing five or six grams placed in a weighed sterile bottle (250 cc) containing broken glass and 100 cc water. The bottle was weighed again and the exact weight of flesh taken determined. This process was repeated on the belly near the ventral fin. A small piece of the gills was also cut off with sterile scissors and placed in a bottle. The fish was then slit open and a piece of the intestine taken from just behind the stomach. (In the case of the king and pink salmon a piece of caecum was also taken and placed in a fifth bottle). The four bottles respectively containing weighed amounts of back flesh, belly flesh, gills and intestines were then shaken vigorously until the sharp pieces of glass had cut the tissues into very small pieces and the bacteria were as uniformly distributed in the liquid as possible. Dilutions were made, using sterile water, in order that the Petri dishes might not be thickly sown, and 1 cc of the diluted liquid placed in a Petri dish. The neutral dextrose agar used was composed of 5 g peptone, 5 g dextrose, 3 g beef extract, 15 g washed agar and 1,000 cc water. It was neutral to phenol red. The plates were incubated at 30° C. for 72 hours and the colonies counted. Other cultural characteristics of the organisms found in the back flesh were studied; among them, growth in anaerobic media (Van Ermengem 1912), and fermentative ability in dextrose and lactose media (Am. Pub. Health Assoc. 1920). Characteristic representative colonies were transferred from the agar plates to agar slants for preservation. Some of the anaerobic tubes were also preserved. The indol producing ability of many of these bacteria was later determined (7, A).

Further physical examination. The condition of the viscera was observed. As a rule no very striking changes took place for several days. Occasionally the viscera was found to be gray or bloody; after several days the viscera usually became soft, frequently broken, and in extreme cases partially liquified. In a few fish the belly walls were broken through so the viscera protruded. The reddening in belly walls is an index of condition. The normal color gradually gave place to a feverish red, which began at the gills and spread backward along the belly walls. The intensity of this abnormal color was greatest near the gills. This is a very characteristic sign of decomposition. It was found in practically every case after the fish had been standing 72 hours or more.

Gas gathers under the membranes. Usually on the fourth day, sometimes on the third, small bubbles were noticed under the membranes which line the belly cavity. By the fifth and sixth days these became very numerous and probably were scattered all through the flesh, producing the condition in canned fish spoken of as "honey-combing."

Loose ribs are an indication of condition. As the fish grew older the belly membranes grew weaker and the flesh became loosened from the bones in such a way that the ribs, during butchering and cleaning, frequently broke loose from the belly walls.

The odor of the gills, viscera, walls of the belly cavity, and flesh in the back, were carefully recorded. The descriptive terms used were: good, lack of odor, stale, taint and putrid; both stale and taint were frequently divided into three degrees, for example, slightly stale, stale, strongly stale. The flesh of all the fish 24 hours old had a good normal fish odor. Frequently the flesh of fish 48 hours old did not possess this characteristic odor nor did it have any odor of decomposition. In such cases we reported this condition as "lack of odor." Stale fish had abnormal odors, with a slight suggestion of decomposition while tainted fish had an unmistakable odor of decomposition; when this was extreme, the fish were spoken of as putrid.

Chemical examination. The amount of indol in the gills, viscera and flesh was determined. The gills, together with the bony structures to which they are attached, were ground in a meat grinder and thoroughly mixed. Under the term "viscera" we placed everything within the belly cavity, including eggs or milt. This was ground and mixed. Whenever the ground gills or viscera amounted to 200 grams, this amount was taken for distillation. When there was

less than 200 g, as large a sample as possible was taken and the weight recorded in order that the indol might be calculated to the uniform basis of 100 g. After the dressed fish was thoroughly washed, portions of the flesh were distilled for indol. The portions selected varied with the different species and are described in the part devoted to each species. These determinations were made according to the method given in 5, G of this paper.

Canning. The remainder of the dressed and cleaned fish was cut transversely into sections of suitable length for half pound (227g) cans. In the case of large fish, some of these sections were big enough to fill two cans; in such cases the section was cut into two nearly equal pieces, and the can receiving the piece without the backbone was marked with a star. The cans were placed in order; and as soon as the covers were clinched on, were marked in such a manner as to identify the species, the individual fish, and the particular section of that fish. For example, the cans obtained from the coho salmon "A" were marked as follows:

CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9
CA2*	CA3*	CA4*		CA6*	CA7*			

In every case from 8 to 8¼ oz. (227-234 g) of fish were placed in each can with about ⅛ oz. (3.5g) of salt. All of the cans, except those containing the king salmon, were exhausted before cooking. For this purpose the covers were clinched on rather loosely and the cans placed in a pressure cooker with the steam issuing freely from the petcock. They remained in the live steam for 12 minutes and were then tightly closed with a hand seamer. All of the cans were cooked at 10 pounds (4.54 kg) pressure (240° F. or 115.5° C.) for 80 minutes, then removed and allowed to cool on the cement floor. A few days after all of the fish of one species were canned, certain cans from each fish were carefully examined according to the method outlined in 2. The cans selected and the reason for their selection are considered in the parts devoted to the several species.

B. *Experimental work on king salmon*

Eighteen king salmon were obtained as they were taken from a trap in the San Juan Islands at 10 a. m., July 30. They were immediately placed in two large fish boxes, covered, and brought on a fish boat to the dock of the San Juan Fish Company at Seattle, where the boxes were stored near the sea end of the dock. The maximum and minimum temperatures in the 7 readings in the fish boxes during

the 6 days of storage were, in centigrade degrees, 21.1 and 15.6, 23.3 and 15.3, 21.1 and 16.1, 19.4 and 15, 19.6 and 15, 19.4 and 15, 20.9 and 14.4; (in Fahrenheit degrees 70 and 60, 74 and 59.5, 70 and 61, 67 and 59, 67.5 and 59, 67 and 59, 69.5 and 58).

Three fish were brought to the laboratory each morning. Indol determinations were made on the gills, viscera and flesh. With a view to obtaining a sample which should represent the whole fish, pieces of flesh, each weighing about 250 grams, were taken from the two ends of the dressed and cleaned fish. These were ground together, mixed, and two portions of 200 g each distilled for indol. The results indicate that an unfortunate selection of flesh was made. The first cut of each fish is likely to contain more indol than any other cut, due to the rapid bacterial invasion from the gills. The last cut also contains more than the average for the fish as a whole. Therefore, since these cuts were selected for the determination of indol in the raw fish, the results were bound to be much higher than those obtained in the cooked salmon using cuts less heavily invaded by bacteria. The indol was determined in three cans from each fish representing the third, sixth and next to the last cuts. The first and last cuts had been used raw, and the second cut (canned) was saved for possible use in a series of food analyses to be made during the winter. The three cans selected gave an idea of the condition of the fish at points near the ventral fins, near the end of the body cavity, and near the tail. (See table 8 and Fig. 2).

C. *Experimental work on pink salmon*

Thirty-six pink salmon taken from a trap in the San Juan Islands about 9 a. m., August 17, were placed in boxes, covered, and brought on a fish boat to the dock of the San Juan Fish Company at Seattle, where the boxes were stored near the sea end of the dock. The maximum and minimum temperatures in the fish boxes during the six days of storage were, in degrees centigrade, 16.1 and 11.7, 16.7 and 12.2, 18 and 13.3, 19.4 and 13.6, 18 and 14.4, 18.9 and 14.4; (in degrees Fahrenheit, 61 and 53, 62 and 54, 64.5 and 56, 67 and 56.5, 64.5 and 58, 66 and 58). (See also table 9 and Fig. 3).

Six fish were brought to the laboratory each morning. Flesh for the indol test consisted of a portion from each end of the fish together with a portion, equal to the two other portions combined, consisting of an entire section of the fish in the region of the dorsal fin. These three portions, weighing altogether about 250 grams, were ground together and mixed. Owing to the small size of the fish, only

TABLE 8. *Average daily decomposition changes in king salmon*

Days	1	2	3	4	5	6
Weight in pounds	14.2	15.8	15.2	12.9	12.3	12.8
Length in inches	31.5	32.8	31.6	31.0	30.0	30.0
Skin	Bright	Bright	Sl. dull	Dull	Dull	Very dull
Scales, adherence	Firm	Firm	Sl. loose	Sl. loose	Loose	Dried on
Eyes	Bright	Sl. sunken	Sunken	Sunken	Sunken-gone	Gone
Gills	Red	Sl. gray	Gray	Sl. gray	Gray, sept-d	Gray, sept'd
Elasticity	Immediate	Sl. slow	Slow	Imp. remains	Imp. remains	Very soft
Viscera	Entire	Entire	Slight	Entire, soft	Entire, soft	Partly gone
Reddening, flesh	None	None	Slight	Slight	Marked	Marked
Gas, membranes	None	None	None	Some	Marked	Marked
Ribs broken loose	No	No	Slightly	Badly	Badly	Badly
Odor, gills	Sl. stale	Sl. taint	Taint	Putrid	Putrid	Putrid
Odor, viscera	Good	Stale	Taint	Taint	Taint	Putrid
Odor, belly walls	Good	Sl. stale	Taint	Putrid	Putrid	Putrid
Odor, back flesh	Good	Sl. stale	Stale	St. taint	Putrid	Putrid
Indol, gills	23.8	8.8	147.0	506.0	1033.0	1500.0
Indol, viscera	3.6	4.7	14.2	10.8	20.3	220.0
Indol, flesh	0.5	1.16	7.83	29.15	93.3	178.8
Bacteria, gills	9,000	22,900	83,000	385,000	205,000	4,290,000
Bacteria, viscera	900	590	225	165	16,000	103,500
Bacteria, belly flesh	0	1,800	23,600	269,500	2,516,000	206,000
Bacteria, back flesh	0	2,000	40,200	17,300	370,000	115,000

Examination of the canned salmon		Examination of the canned salmon	
Liquid, turbidity	Normal	Normal	Sl. turbid
Odor	Good	Stale	Sl. taint
Texture	Firm	Sl. soft	Soft
Color	Good	Average	Abnormal
Reddening, flesh	None	V. slight	Marked
"Honeycombing"	None	Slight	Marked
Biting taste	None	Slight	Marked
Ribs broken loose	No	No	Marked
Indol	0.39	2.08	Badly
Bact. examination	8 sterile	8 sterile	35.1
			9 (all) sterile

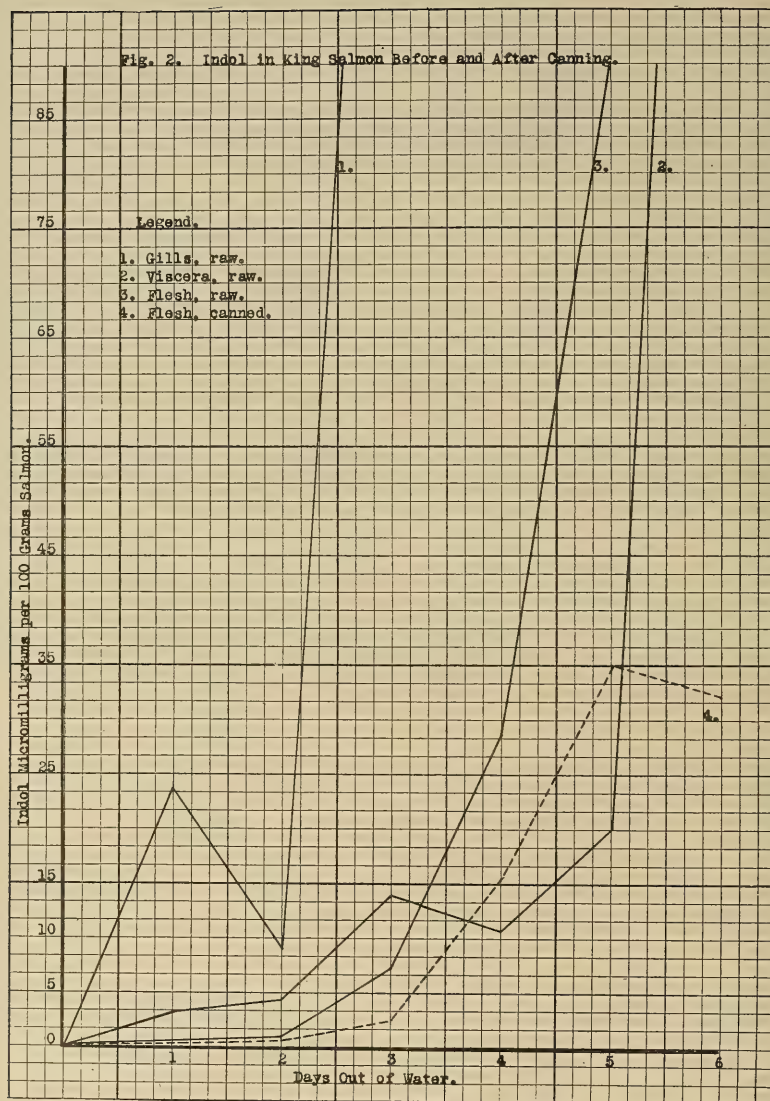


Fig. 2. Indol in king salmon before and after canning.

TABLE 9. Average daily decomposition changes in pink salmon

Days	1	2	3	4	5	6
Weight in pounds	4.1	3.5	3.5	4.0	4.3	3.4
Length in inches	21.5	20.9	20.7	22.0	22.6	20.7
Skin	Bright	Bright	V. sl. dull	Sl. dull	Sl. dull	Dull
Scales, adherence	Firm	Sl. loose	Loose	Sl. loose	Sl. loose	Sl. loose
Eyes	Bright	Sl. bloody	Bloody	Sunken	Sunken	Gone
Gills	Red	Sl. gray	Sl. gray	Gray	Gray	Gray, sept'd
Elasticity	Immediate	Sl. slow	Slow	Very slow	Imp. remains	Soft
Viscera	Entire	Entire	Entire	Entire, soft	Sl. broken	Broken
Reddening, flesh	None	None	Slight	Slight	Marked	Marked
Gas, membranes	None	None	None	None	Some	Marked
Ribs broken loose	No	No	Slightly	Badly	Badly	Badly
Odor, gills	Sl. stale	Sl. stale	Stale	Taint	Putrid	Putrid
Odor, viscera	Good	Good	Sl. stale	Sl. taint	Sl. taint.	Taint
Odor, belly walls	Good	Good	V. sl. stale	Sl. taint	St. taint.	Taint
Odor, back flesh	Good	Good	Good	Stale	Taint	Taint
Indol, gills	0.56	1.8	5.5	378.0	423.0	918.0
Indol, viscera	0.15	0.17	0.23	2.7	1.4	34.0
Indol, flesh	0.1	0.18	0.25	2.65	23.6	90.0
Bacteria, gills	30,500	49,500	1,314,500	2,157,500	70,958,000	37,891,000
Bacteria, viscera	226	2,130	45,500	65,000	2,581,000	970,000
Bacteria, belly flesh	1,450	15,500	44,300	21,500	2,490,000	353,000
Bacteria, back flesh	85	17,100	30,400	8,500	448,000	32,800
Examination of the canned salmon						
Liquid, turbidity	Normal	Normal	Sl. turbid	Sl. turbid	Turbid	Turbid
Odor	Good	Good	Stale	Sl. taint	Taint	Putrid
Texture	Firm	Sl. soft	Sl. soft	Soft	Soft	Soft
Color	Average	Average	Average	Abnormal	Abnormal	Abnormal
Reddening, flesh	None	None	Slight	Marked	Marked	Marked
"Honeycombing"	None	None	Slight	Marked	Marked	Marked
Biting taste	None	None	Slight	Marked	Marked	Marked
Ribs broken loose	No	No	Yes	Badly	Badly	Badly
Indol	0.35	0.33	0.53	1.92	7.77	15.66
Bact. examination	5 sterile	6 sterile	6 sterile	5 sterile	6 (all) sterile	3 sterile

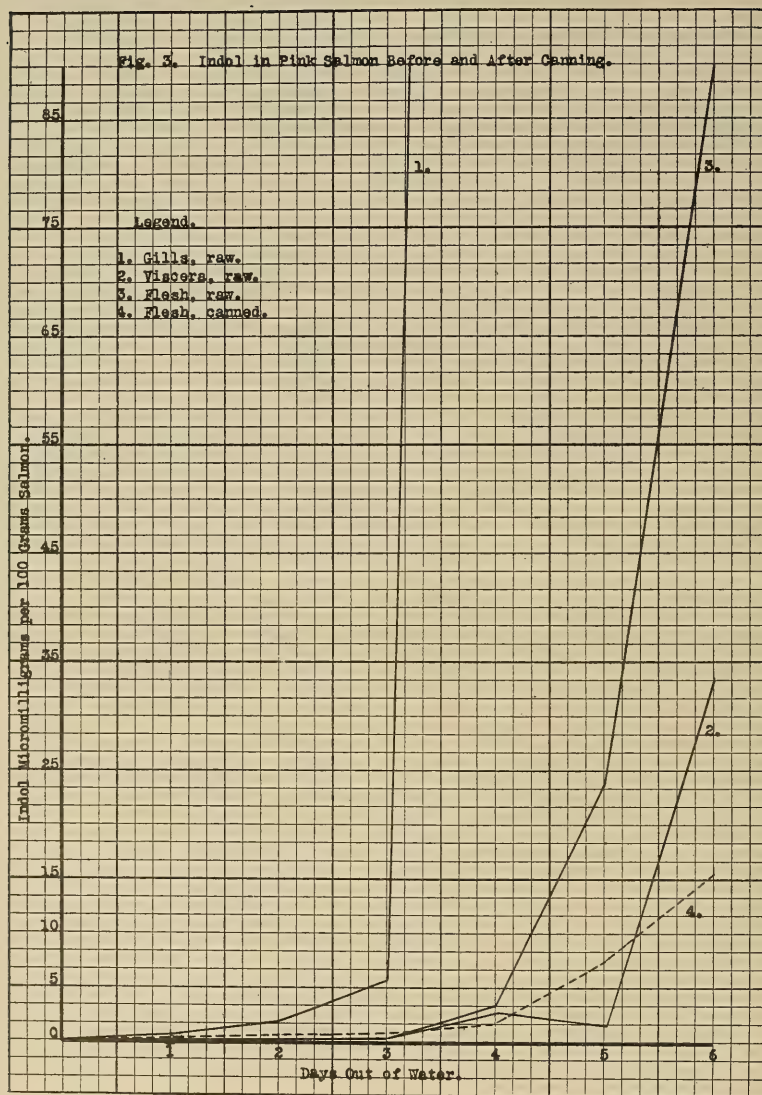


Fig. 3. Indol in pink salmon before and after canning.

one indol determination was made on the flesh. The results again indicated that the portions of the flesh were not wisely selected.

D. *Experimental work on sockeye salmon*

Thirty sockeye salmon were obtained as they were taken from a trap in the San Juan Islands about 9 a.m., September 3. They were immediately placed in boxes on the deck of the fish boat, covered, and brought to Seattle. Upon arrival early in the morning of September 4, five were taken at once to the laboratory, and the rest stored on the dock. The maximum and minimum temperatures in the fish boxes during the 6 days of storage were, in centigrade degrees, 16.7 and 12.2, 16.7 and 10, 15.6 and 13.3, 17 and 11.7, 16.7 and 11.1, 16.7 and 11.7; (in Fahrenheit degrees, 62 and 54, 62 and 50, 60 and 56, 62.5 and 53, 62 and 52, 62 and 53). (See table 10 and Fig. 4).

Five sockeyes were brought to the laboratory each morning. Flesh for the indol determination was selected in such a way as to show whether the cooking of the canned fish increased or diminished the amount of indol present. The first transverse section of the fish (called the first cut) consisting of about one pound (454 g) was divided as equally as possible into two parts, in one of which the indol was determined at once, and in the other after canning. A section of similar weight, cut from the fish just in front of the dorsal fin (usually the third or fourth cut) was treated in an exactly similar manner. The results gave a much closer agreement between the raw and cooked salmon than was obtained with the king and the pink salmon.

E. *Experimental work on coho salmon*

Twenty four coho salmon were obtained as they were taken from a trap in the San Juan Islands about 9 a.m., September 3. They were immediately placed in boxes on the deck of the fish boat, covered, and brought to Seattle. Upon arrival, early in the morning of September 4, four were taken at once to the laboratory, and the rest stored on the dock. The maximum and minimum temperatures in the fish boxes during the 6 days of storage were, in centigrade degrees, 16.7 and 12.2, 16.7 and 10, 15.6 and 13.3, 17 and 11.7, 17 and 11.7, 16.7 and 11.1, 16.7 and 11.7; (in Fahrenheit degrees, 62 and 54, 62 and 50, 60 and 56, 62.5 and 53, 62 and 52, 62 and 53).

Four coho salmon were brought to the laboratory each morning. Flesh for the indol determination was selected as in the case of the sockeye salmon. The results gave a much closer agreement between the raw and the cooked salmon than was obtained with the king and the pink salmon. (See table 11 and Fig. 5).

TABLE 10. *Average daily decomposition changes in sockeye salmon*

Days	1	2	3	4	5	6
Weight in pounds	6.35	5.9	6.25	5.35	5.55	6.2
Length in inches	25.0	23.9	24.8	22.8	23.5	24.8
Skin	Bright	Bright	Sl. dull	Dull	Dull	Very dull
Scales, adherence	Firm	Sl. loose	Loose	Loose	Loose	Very loose
Eyes	Bright	Sl. bloody	Bloody	Sunken, gray	Sunken, gone	Sunken, gone
Gills	Bright red	Sl. gray	Sl. gray	Gray	Gray	Gray, sept'd
Elasticity	Immediate	Sl. slow	Slow	Imp. remains	Imp. remains	Soft
Viscera	Entire	Entire	Entire	Entire	Entire, gray	Sl. broken
Reddening, flesh	No	No	Slight	Slight	Marked	Marked
Gas, membranes	No	No	No	Slight	Marked	Marked
Ribs broken loose	No	No	Slightly	Slightly	Badly	Badly
Odor, gills	Good	Stale	Taint	Taint	Taint	Putrid
Odor, viscera	Good	Good	Sl. stale	Sl. stale	Stale	St. stale
Odor, belly walls	Good	Good	Stale	St. stale	Sl. taint	Taint
Odor, back flesh	Good	Good	Sl. stale	Stale	Sl. stale	Sl. taint
Indol, gills	1.6	23.1	156.5	139.0	266.0	591.0
Indol, viscera	0.16	0.1	0.54	1.8	8.5	14.2
Indol, first cut	0.1	0.36	2.4	9.7	33.0	38.2
Indol, dorsal cut	0.0	0.24	1.4	4.2	17.3	25.2
Bacteria, gills	172,000	74,000	7,500,000	4,960,000	25,165,000	6,130,000
Bacteria, viscera	2,700	670	491,000	18,900	278,000	120,900
Bacteria, belly flesh	8	410	520	7,660	210,000	1,650,000
Bacteria, back flesh	6	29	89	6,790	17,100	133,200
Examination of the canned salmon						
Liquid, turbidity	Normal	Normal	Sl. turbid	Sl. turbid	Turbid	Turbid
Odor	Good	Good	Stale	St. stale	Taint	Taint
Texture	Firm	V. sl. soft	Sl. soft	Sl. soft	Soft	Soft
Color	Good	Good	Average	Abnormal	Abnormal	Abnormal
Reddening, flesh	None	None	V. slight	Sl. marked	Marked	Marked
"Honeycombing"	None	None	V. slight	Marked	Marked	Marked
Biting taste	None	None	V. slight	Marked	Marked	Marked
Ribs broken loose	No	No	No	Slightly	Badly	Badly
Indol	0.2	1.9	2.45	5.05	19.2	28.3
Bact. examination	Sterile	Sterile	Sterile	Sterile	Sterile	Sterile

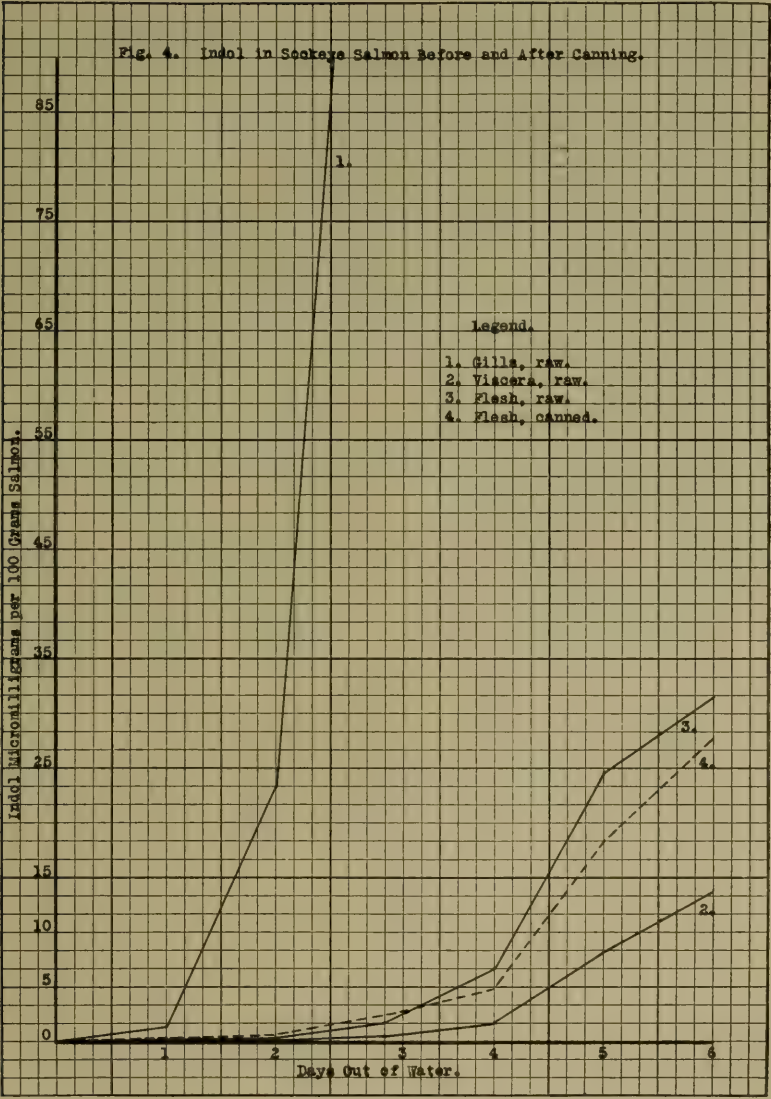


Fig. 4. Indol in sockeye salmon before and after canning.

TABLE 11. Average daily decomposition changes in coho salmon

Days	1	2	3	4	5	6
Weight in pounds	11.5	9.4	8.8	9.0	9.5	12.0
Length in inches	27.5	26.2	26.2	25.8	26.2	28.5
Skin	Bright	Bright	Sl. dull	Dull	Dull	Dull
Scales, adherence	Firm	sl. loose	Sl. loose	Loose	Loose	Very loose
Eyes	Bright	Sl. bloody	Sunken, gray	Sunken, gone	Sunken, gone	Sunken, gone
Gills	Bright	Sl. gray	Sl. gray	Gray, sept'd	Gray, sept'd	Gray, sept'd
Elasticity	Immediate	Sl. slow	Slow	Imp. remains	Imp. remains	Very soft
Viscera	Entire	Entire	Entire	Entire	Broken, gray	Sl. broken
Reddening, flesh	None	None	Slight	Yes	Marked	Marked
Gas, membranes	None	None	No	Sl. to badly	Marked	Marked
Ribs broken loose	No	No	Slightly	Taint	Badly	Badly
Odor, gills	Good	Sl. stale	Taint	Stale	St. taint	St. taint
Odor, viscera	Good	Good	Sl. stale	Stale	Sl. taint	Sl. taint
Odor, belly walls	Good	Sl. stale	Stale	St. stale	Taint	Taint
Odor, back flesh	Good	Good	Sl. stale	Stale	Sl. stale	Sl. taint
Indol, gills	0.3	16.5	11.5	103.0	263.0	196.0
Indol, viscera	0.1	0.1	0.7	1.0	0.6	2.1
Indol, first cut	0.1	0.25	1.3	6.2	15.0	31.6
Indol, dorsal cut	0.0	0.1	0.8	3.8	15.7	14.5
Bacteria, gills	1,840	30,000	636,700	412,500	3,900,000	597,500
Bacteria, viscera	237	49	11,700	15,900	436,000	1,100
Bacteria, belly flesh	20	6,570	460	400	67,500	19,300
Bacteria, back flesh	0	3,000	1,380	190	86,200	24,500
Examination of the canned salmon						
Liquid, turbidity	Normal	Normal	Sl. turbid	Turbid	Turbid	Turbid
Odor	Good	Good	stale	Taint	Taint	Taint
Texture	Firm	Firm	Sl. soft	Soft	Soft	Soft
Color	Good	Good	Good	Abnormal	Abnormal	Abnormal
Reddening, flesh	None	None	Slight	Marked	Marked	Marked
"Honeycombing"	None	0.4	Slight	Marked	Marked	Marked
Biting taste	None	None	Slight	Marked	Marked	Marked
Ribs broken loose	No	None	No	Yes	Yes	Yes
Indol	0.22	No	2.1	5.9	16.4	20.1
Bact. examination	8 sterile	8 sterile	8 sterile	6 sterile	8 sterile	8 (all) sterile

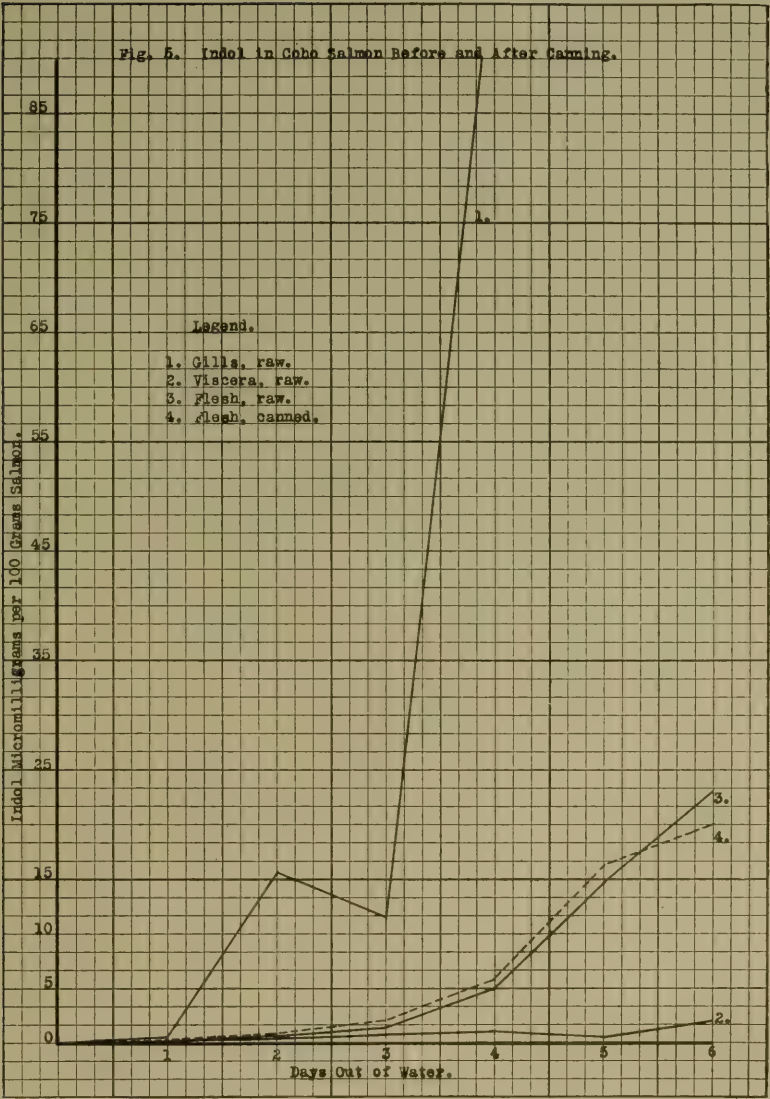


Fig. 5. Indol in coho salmon before and after canning.

F. *Experimental work on chum salmon*

Thirty chum salmon were obtained from a trap at Meadow Point, near Seattle, about 8 a.m., October 22. They were brought to Seattle on a scow and unloaded at the dock about 10 a.m. All of the fish were taken at once to the laboratory and stored in a box having several shelves so that there might not be too much pressure on any of the fish. For two days the box was kept in the laboratory near an open window and was then moved outside. The maximum and minimum temperatures in the box during the 6 days of storage were, in centigrade degrees, 20.6 and 15, 21.7 and 16.7, 22.2 and 11.1, 14.4 and 11.1, 17.8 and 11.1, 13.3 and 10.6; (in Fahrenheit degrees, 69 and 59, 71 and 62, 72 and 52, 58 and 52, 64 and 52, 56 and 51.)

Five fish were used each day. Flesh for the indol determination was selected as in the case of the sockeye salmon. The results were somewhat higher in the fish out of water 48 hours than the results obtained from any of the other species, probably due to the higher storage temperature. (See table 12 and Fig. 6).

Indol in raw salmon at different stages of decomposition was quantitatively determined in 138 fish. Two determinations were made on each fish, with the exception of the pink salmon, on which but one was made. In all, 229 determinations were made. Although the results have already been given they are rearranged here to bring out the correlation between indol content and odor; and for comparison with the results obtained with the same salmon when canned (table 14) as well as those obtained with commercial cans of salmon (table 15). The odor on which table 13 was based was the odor of the belly cavity.

As the odor of decomposition increased in the belly cavity, the amount of indol in the flesh also increased. The percentage of determinations having 1.5 mmg indol or more per 100 g of salmon increased in proportion to the odor of decomposition. The agreement between the results given here and those given in table 14 is very good. The terms used here to designate odor have been defined in 2. Although it is recognized that stale fish is of inferior quality, it is usually considered as not unfit for food. In this and other tables, therefore, those fish or cans of fish classed as stale have been designated as "passed by odor as fit for food," while tainted fish or cans of fish have been designated as "not passed by odor."

Indol in experimental packs of canned salmon was quantitatively determined in 269 cans of the five species. The results have already

TABLE 12. *Average daily decomposition changes in chum salmon*

Days	1	2	3	4	5	6
Weight in pounds	11.5	12.2	10.9	9.1	10.1	10.8
Length in inches	29.4	30.0	29.1	27.8	28.3	29.7
Skin	Bright	Bright	Sl. dull	Dull	Dull	Very dull
Scales, adherence	Firm	Sl. loose	Sl. loose	Loose	Loose	Very loose
Eyes	Bright	Sl. bloody	Sunken, gray	Sunken, gray	Sunken, gone	Sunken, gone
Gills	Bright red	Sl. gray	Gray	Gray	Gray-green	Gray-green
Elasticity	Immediate	Quickly	Slowly	Slowly	Very slow	Imp. remains
Viscera	Entire	Entire	Sl. gray	Sl. broken	Gray	Sl. broken
Reddening, flesh	None	None	Slight	Marked	Marked	Marked
Gas, membranes	None	None	Slight	Marked	Marked	Marked
Ribs broken loose	No	No	Slightly	Slightly	Sl. to badly	Badly
Odor, gills	Good	St. stale	Taint	Putrid	Putrid	Putrid
Odor, viscera	Good	Good	Stale	Taint	Taint	St. taint
Odor, belly walls	Good	Sl. stale	Stale	Sl. taint	Taint	St. taint
Odor, back flesh	Good	Sl. stale	Stale	St. stale	Taint	St. taint
Indol, gills	0.8	15.9	66.4	197.0	938.0	866.0
Indol, viscera	0.4	1.3	3.5	10.2	44.0	126.0
Indol, first cut	0.26	1.4	8.0	17.7	58.0	91.0
Indol, dorsal cut	0.1	0.5	3.8	6.9	19.7	42.0
Bacteria, gills	180,800	3,819,000	85,960,000	41,000,000	616,000,000	1,123,000,000
Bacteria, viscera	1,490	33,720	167,000	929,000	1,153,000	8,275,000
Bacteria, belly flesh	16	1,570	57,500	116,100	1,944,000	2,538,000
Bacteria, back flesh	2	296	29,500	32,500	2,512,000	1,502,000
Examination of the canned salmon						
Liquid, turbidity	Normal	Normal	Sl. turbid	Turbid	Turbid	Turbid
Odor	Good	V. sl. stale	St. stale	Taint	St. taint	Putrid
Texture	Firm	Firm	Sl. soft	Soft	Soft	Soft
Color	Good	Good	Average	Abnormal	Abnormal	Abnormal
Reddening, flesh	None	Very slight	Slight	Marked	Marked	Marked
"Honeycombing"	None	None	Slight	Marked	Marked	Marked
Biting taste	None	None	Slight	Marked	Marked	Marked
Ribs broken loose	No	No	V. slightly	Slightly	Badly	Badly
Indol	0.25	1.8	9.6	24.6	51.8	84.4
Bact. examination	9. sterile	9 sterile	9 sterile	9 sterile	10 sterile	10 (all) sterile

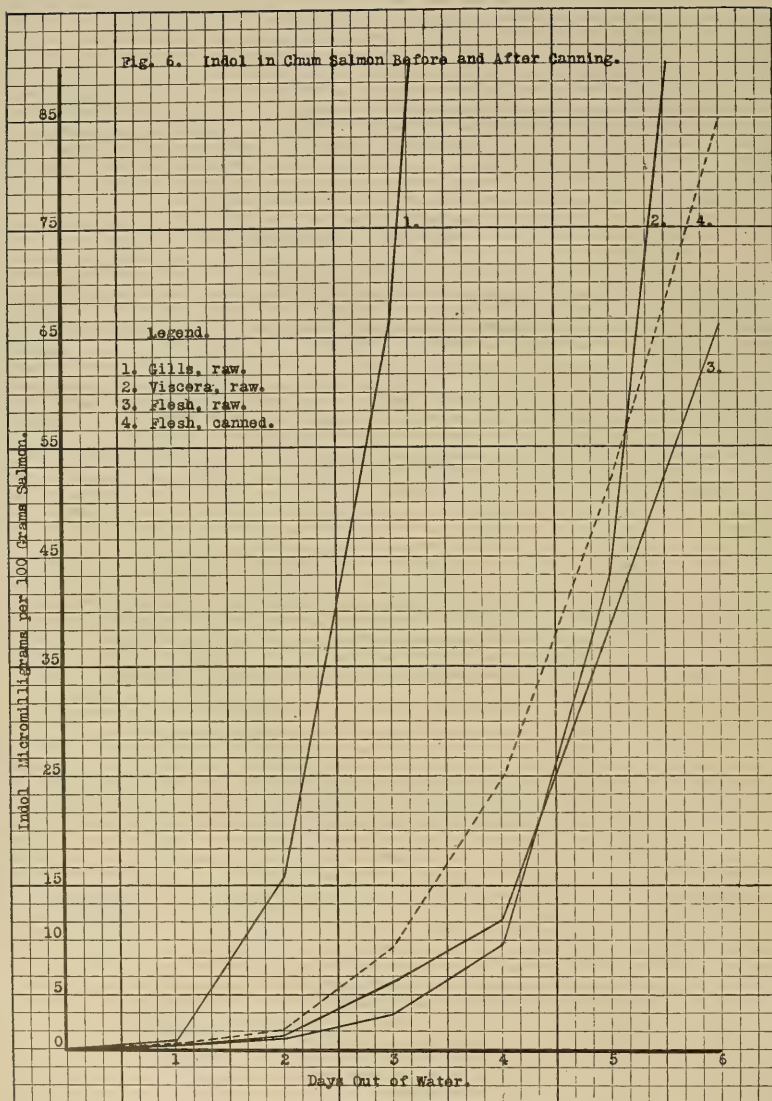


Fig. 6. Indol in chum salmon before and after canning.

TABLE 13. *Correlation between indol and odor in raw salmon.*

Indol mmg per 100 g salmon	Number of cuts classed by odor as—					Total
	Good	Slightly stale	Stale	Strongly stale	Tainted or putrid	
0.1—0.5	58	10	3	0	0	71
0.6—1.0	4	10	4	0	0	18
1.1—1.4	0	1	2	0	0	3
1.5—	0	10	21	12	94	137
Total	62	31	30	12	94	229
1.5—	0.0%	32.2%	70.0%	100.0%	100.0%	59.8%

	Reported as good.	Reported as stale.
0.1—0.5	68	3
0.6—1.0	14	4
1.1—1.4	1	2
1.5—	10	33
Total	93	42
1.5—	10.7%	78.5%

	Passed by odor as fit for food.	Not passed by odor.
0.0—0.5	71	0
0.6—1.0	18	0
1.1—1.4	3	0
1.5—	43	94
Total	135	94
1.5—	31.8%	100.0%

been given in the previous pages of this section but are rearranged here in order to bring out the correlation between indol content and odor. Furthermore, comparison with the raw salmon used, and with commercial packs of salmon, can be more easily made.

The results show an increasing percentage of cans containing 1.5 mmg (or more) per 100 g of salmon, with an increase in the odor of decomposition. A few of the cans classed as good (3.9%) contained as much as 1.5 mmg while practically all of those classed as strongly stale or tainted contained this amount or more. The percentage of cans containing indol to the extent of 1.5 mmg or more is much higher than in the case of commercial cans of salmon (table 15). The agreement between the raw salmon (table 13) and the experimental cans of salmon prepared from them (table 14) is very good. Why the commercial cans contained less indol is not known. It may be that the bacterial flora was different. Pugsley (date?) carried on a decomposition experiment similar to the one described in this section, using chum salmon which had been in cold storage for three months,

TABLE 14. *Correlation between indol and odor in experimental cans of salmon.*

Indol mmg per 100 g salmon	Number of cans classed by odor as					Total
	Good	Slightly stale	Stale	Strongly stale	Tainted or putrid	
0.0—0.5	46	4	2	1	0	53
0.6—1.0	24	6	8	0	1	39
1.1—1.4	3	2	3	0	1	9
1.5—	3	7	22	24	112	168
Total	76	19	35	25	114	269
1.5—	3.9%	36.8%	62.8%	96.6%	98.6%	62.4%

	Reported as good.	Reported as stale.
0.0—0.5	50	3
0.6—1.0	30	8
1.1—1.4	5	3
1.5—	10	46
Total	95	60
1.5—	10.5%	65.9%

	Passed by odor as fit for food.	Not passed by odor.
0.0—0.5	53	0
0.6—1.0	38	1
1.1—1.4	8	1
1.5—	56	112
Total	155	114
1.5—	36.1%	98.6%

and some which had not been in cold storage at all. The results were entirely different. As stated in 7, A, apparently all of the indol forming bacteria had been killed during cold storage, and both the raw salmon and the canned salmon prepared from them contained almost no indol even after five or six days' storage at room temperature; whereas the raw salmon which had not been in cold storage, and the canned salmon prepared from them, contained large amounts of indol after the third day. This example shows what a big variation in decomposition products may result from different bacterial floras.

Indol in commercially canned salmon was quantitatively determined in 544 cans during a period of ten months. These cans were drawn from 168 separate parcels of salmon, many of which were of rather poor quality. For this reason the proportion of stale and tainted cans is very much higher than is found in the average parcel of canned salmon. In all 1,897 cans were opened for examination and 544 tested for indol. In some of the samples the first 12 cans opened

were used for the indol test without regard to their condition, while in many samples only those which were classed as stale or tainted were used. This is an additional reason why the proportion of stale and tainted cans is so large in table 15.

TABLE 15. *Correlation between indol and odor in commercial cans of salmon.*

Indol mmg per 100 g salmon	Number of cans classed by odor as—				
	Good	Slightly stale	Stale	Strongly stale	Tainted or putrid
0.0—0.5	110	45	48	5	4
0.6—1.0	77	55	77	12	15
1.1—1.4	7	7	8	3	2
1.5—	11	11	18	7	22
Total	205	118	151	27	43
1.5—	5.36%	9.3%	11.9%	25.9%	51.1%

	Reported as good.	Reported as stale.
0.0—0.5	155	53
0.6—1.0	132	89
1.1—1.4	14	11
1.5—	22	25
Total	323	178
1.5—	6.81%	14.0%

	Passed by odor as fit for food.	Not passed by odor.
0.0—0.5	208	4
0.6—1.0	221	15
1.1—1.4	25	2
1.5—	47	22
Total	591	43
1.5—	9.38%	51.1%

The results show an increasing percentage of cans containing 1.5 mmg (or more) of indol per 100 g of salmon, with an increase in the odor of decomposition. However, a few (5.36%) of the cans classed as good would be condemned on the basis of 1.5 mmg of indol, while nearly half (48.9%) of those condemned by odor would be passed by the indol test. As stated in 6, G, almost none of the cans (from our experimental packs) prepared from fish out of water 48 hours or less, gave a test for more than 1.5 mmg indol, and in those cases the raw fish showed signs of decomposition. It is evident, therefore, that although these few cans had an odor classed as good, considerable decomposition had already taken place. As pointed out in 7, A, many bacteria do not produce indol, although they may bring about a putre-

faction of the fish. This may explain why such a large percentage of tainted commercial canned salmon contained less than 1.5 mmg indol. The experimental pack of salmon as shown in table 14 contained more indol than the commercial cans examined. The difference may be due to a difference in bacterial flora between the waters of Puget Sound, where the fish for the experimental pack were caught; and the waters of Alaska, where most of the fish for these commercial cans were caught. There may be a larger percentage of indol formers in the flora of the waters of Puget Sound.

In conclusion it may be said that when indol is found in amounts of 1.5 mmg or over per 100 g it is certain that a considerable amount of decomposition has taken place. However, we cannot safely accept the absence of indol as conclusive evidence of the absence of decomposition. In other words, a positive test has considerable value in judging the condition of canned salmon as regards decomposition, while a negative test is of little value.

G. Discussion of the results

The results obtained in the experimental work on the five species of salmon have been summarized for each species, in tables and figures, in the previous pages of part 6. Furthermore, all of the conditions and changes studied during this investigation have already been discussed to some extent in the general outline of the experiment.

The physical results on each of the species are very similar. All of the indications of decomposition increased in intensity from day to day, and although there were individual fish or cans which did not follow the general rule, the averages for the fish examined from day to day showed a consistent and regular increase in each of these indications; this is shown in the tables giving the average daily decomposition changes. The chum salmon showed more rapid increase in decomposition changes than the others, and this was probably due to the higher temperature at which they were stored during the first two days. In the pink salmon, on the other hand, decomposition proceeded much more slowly; and this again was apparently due to the somewhat lower temperature prevailing at the beginning of the experiments on pink salmon.

The chemical results from the different species are also similar, but here, too, the influence of the storage temperature is very marked, some of the chum salmon giving a test for 1.5 mmg or more of indol at the end of 48 hours, while none of the pinks gave a test for this amount until after 96 hours. In the case of all the species, however,

the amount of indol increased from day to day; and although there were some determinations both of the raw and of the canned salmon which did not follow the general rule, yet the averages for the fish examined each day showed a rather consistent increase. The curves which are given for each species show that during the first two or three days little indol is found; but after it begins to appear in noticeable quantities, the increase is very rapid, or, in other words, the curve bends sharply upward. The curves showing the results obtained from the gills do not show the pronounced initial lag shown by the curves for the flesh, either raw or canned, and this is no doubt due to the fact that the gills contain many bacteria at the beginning of the storage period, while the flesh is free from bacteria. Indol production, therefore, may start at once in the gills, but not in the flesh until after it has been invaded by the bacteria. This invasion, as shown by the bacteriological results, is rather slow for the first 48 hours; this agrees with the indol production. The curves for the viscera in most of the species show a longer initial lag than those for either the flesh or the gills; and the amount of indol in the advanced stages of decomposition of the viscera was less in nearly every case than in the flesh. It may be that the viscera are a poor medium for the growth of bacteria and the formation of indol. Furthermore, the presence of the milt and roe (which are very slow in showing signs of decomposition) in the ground viscera served to dilute the more easily putrescible parts, such as the intestines, stomach, heart, etc., and thus lowering the percentage of indol. Since the bacteriological samples were taken from the intestines only, while the chemical samples included the entire contents of the belly cavity, is it not surprising that the results do not agree very well.

The curves given in Figs. 2 and 3 for king and pink salmon respectively, do not show a good agreement between the raw and the cooked flesh; the raw flesh has more indol than the cooked. As pointed out elsewhere, this is due not to loss of indol in the canning process, but to an unfortunate choice of samples for this determination; the raw salmon used was taken from those parts of the fish most heavily invaded by bacteria, while the canned salmon used represented parts much less heavily invaded. The curves given in Figs. 4, 5, and 6, for sockeye, coho and chum salmon, respectively show very good agreement between the raw and the cooked flesh. This is due to the fact that the raw and canned flesh used for the indol determination came from the same cut of the fish, one half being used raw and the other half canned.

The variation in the amount of indol found in different parts of

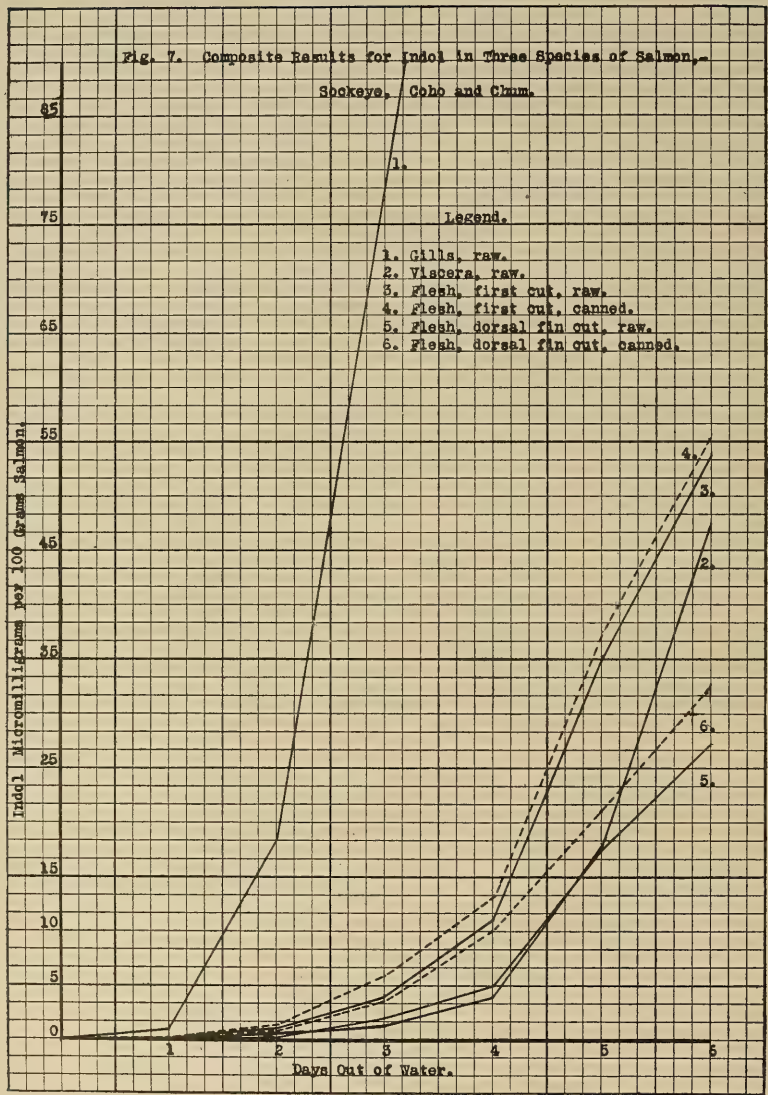


Fig. 7. Composite results for indol in sockeye, coho and chum salmon.

the same fish is shown in Fig. 7, which gives composite curves showing the results from the first cut and those from a cut near the dorsal fin. The first cut contains more indol because it is near the gills where it is quickly and heavily invaded by bacteria. These composite curves show excellent agreement between the results from the raw and the cooked flesh, especially in the first cut. Why slightly more indol was found in the cooked than in the raw flesh is not clear, but it may be that the cooking process serves to break down the cell structure, liberating the indol, resulting in a more rapid and complete distillation.

Figure 8 shows the results from the raw flesh of the five species. The difference in temperature prevailing at the time these different species were under examination may have been partly responsible for the difference in the results obtained. The maximum and minimum temperatures were taken each day, and these have already been given; but for convenience, curves showing the mean temperatures from day to day are also given in Fig. 8. Inspection of these curves shows that the king salmon was stored at the highest average temperature and the coho and sockeye at the lowest. The king salmon contained the most indol, while the sockeye and coho had the least, with the exception of the pink, which had less during the first four days. The chum salmon curve follows that of the king very closely at first; but the rate of increase falls off, and the falling off is coincident with a sharp lowering of the temperature when the chum salmon were moved from the laboratory to the outside of the building. The low temperature prevailing at the beginning of the experiments with pink salmon is no doubt partially responsible for the small amount of indol found during the first four days; while the increasingly higher temperatures toward the end of the storage period, together with their small size, may account for the rather rapid increase in indol content during the fifth and sixth days. Since the sockeye and coho salmon experiments were made at the same time, the temperature was not responsible for the difference in indol content. It is possible that this difference is due to the difference in the average size of the two species; the coho salmon is much larger, so the bacteria would be somewhat longer in invading all parts of the flesh, and the proportion of indol in the flesh would be less. Temperature is without doubt a big factor in the spoilage of salmon, and the correlation between it and indol formation is quite apparent from a study of the curves in Fig. 8.

Skatol was not found in any of the raw salmon, nor in the cans prepared from them; but indol was found in every test made on salmon which had been held for 48 hours or more. An organism capable of

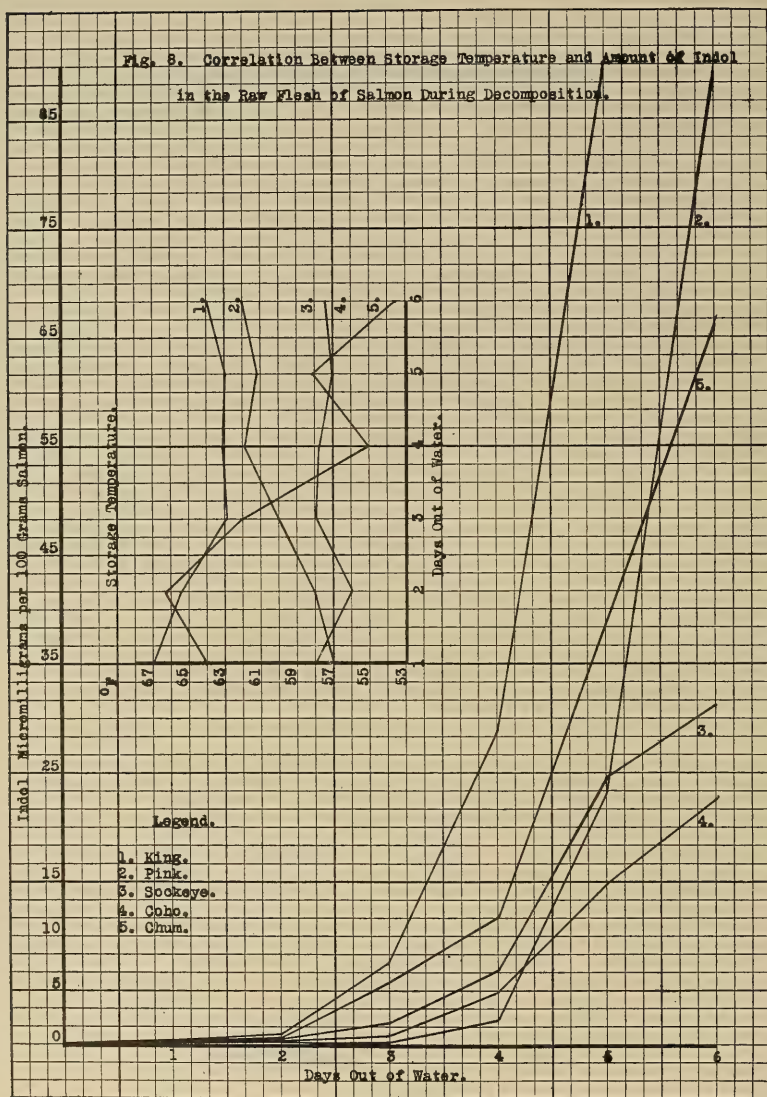


Fig. 8. Correlation between storage temperature and amount of indol.

producing skatol was however isolated from the king salmon (7, A).

The bacteriological results are very similar to the chemical results, as might be expected, since the chemical substances formed during decomposition are due to the bacteria present. However, as is pointed out in a later section, not all bacteria produce indol, and for this reason the correlation between numbers of bacteria and indol content may or may not be a close one. The number of bacteria increased from day to day in each of the parts of the fish examined; gills, viscera, back flesh and belly flesh. The gills contained a large number of bacteria when the fish were drawn from the water, while it is probable that the other three parts of the fish examined were free from bacteria. At the end of 24 hours the gills usually contained several thousand bacteria per gram while the viscera sometimes and the flesh usually was sterile or contained very few bacteria.

The correlation between the bacteriological results and the chemical results has been shown in the tables containing the average daily decomposition changes of the five species of salmon. For convenience these daily averages have been composited and the results expressed in curves in Fig. 9. In general the correlation is very good; the curves for indol and for bacteria, in both the gills and the viscera, have very nearly the same shape throughout. It will be noted that the belly flesh is more rapidly invaded than the back, and this is probably due to the fact that the layer of flesh in the belly is much thinner than that in the back. The curve for indol in the raw flesh falls between the curves for bacteria in the belly and back flesh, due to the fact that the raw flesh was made up of both belly and back flesh. The sharp falling off in the number of living bacteria between the fifth and sixth day indicates that the media had become unfavorable, at least to some of the species of bacteria, with a consequent heavy mortality.

From a consideration of the results obtained in this study of the decomposition of salmon, and from results which were later obtained in a study of the intestinal bacterial flora, it is believed that the spoilage of fish taken while on the spawning migration proceeds primarily from the outside of the fish and not from the digestive tract. Bacteria invade the flesh from the gills, the anus, and the skin. In the case of fish caught while still feeding (by trolling), spoilage will proceed from both the outside and the inside. Correlations between the odor and the indol content in the raw salmon, in our experimentally packed salmon, and in commercial packs of salmon, have been given on previous pages. Correlations between the bacteriological and the chemical results have also been shown, as well as between the indol content and the temperature of spoilage. Correlations between the tempera-

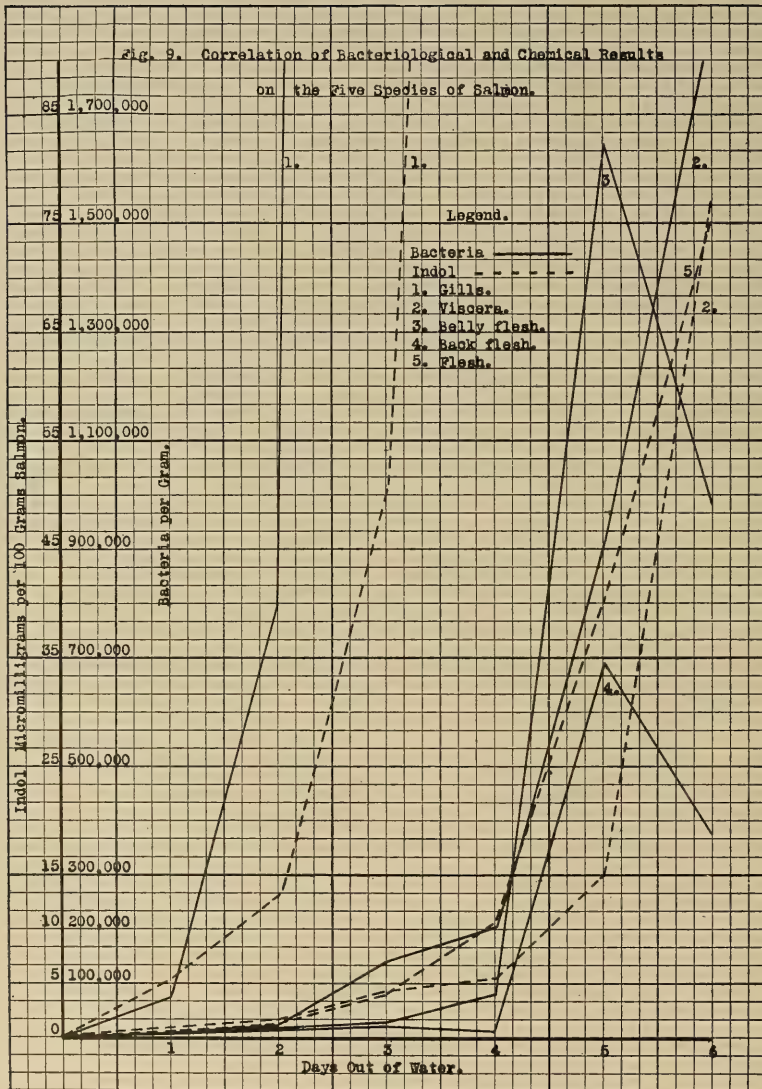


Fig. 9. Correlation of bacteriological and chemical results.

ture, the indol content, the number of bacteria present, and various signs of decomposition, such as reddening, texture, etc., might be shown in a similar way; but enough has been written to show that the physical, chemical and bacteriological changes proceed simultaneously and approximately parallel, and that the rate is dependent to a large extent upon the temperature. Although the indol test cannot supplant the examination by odor and other physical signs of decomposition, it forms, nevertheless, a very useful check on the other methods of examination, and affords considerable information as to the history of the salmon under inspection.

7. FORMATION OF INDOL BY VARIOUS MEANS

A. *Indol formation by bacteria*

So many cases were encountered in the study of both raw and canned salmon, in which the odor and the indol content were not in accord, that it seemed advisable to make a study of the bacteria which Dr. C. R. Fellers had isolated during the above investigation, to ascertain how large a percentage of them would produce indol in Dunham's peptone solution. In many cases cans classed by odor as "slightly stale", or even as "good", were found to contain as much indol as other cans which were classed as "tainted"; and on the other hand, cans having a tainted odor sometimes did not give a test for either indol or skatol. Since the foul odor must be due to bacterial decomposition, it is evident that spoilage may proceed without the formation of these specific products. Rettger (1906, 1908), in his work on putrefaction, states that true putrefaction is due to strict anaerobes alone, and indol is seldom formed. Furthermore, *Bacillus botulinus*, a strict anaerobe, was inoculated for experiment into some sterile cans of salmon, then the cans exhausted and again sealed. After standing about two days at the temperature of the laboratory, the cans showed a pronounced swelling; on opening, they were found to possess a putrid odor; in fact, the contents of each can was in a liquid condition and bubbling with gas. No indol was found in any of these cans.

The bacteria used in the determination of indol production were derived from the following five sources: 1. Isolated from the five species of salmon (raw) during the study of decomposition. 2. Isolated from commercially canned salmon. 3. Taken by the writer from raw salmon (gills, viscera, flesh and pugh marks) at various points in central and southeastern Alaska. 4. Taken by Dr.

C. R. Fellers from four species of salmon (raw) at Blaine, Washington. Taken from points just in front of and just behind the stomach, to ascertain whether the intestinal tract of salmon on the spawning migration is sterile. A few cultures were also made from the gills and pugh marks. 5. Taken from chum salmon which had been held frozen in cold storage for three months.

The method of experimentation was to inoculate the bacteria into 10 cc of Dunham's peptone media and incubate at 37° C. for one week. The tube was then emptied into a 250 cc Fry flask and washed out with 40 cc of water. A current of steam was passed through and 100 cc of distillate collected. This was acidified with 2 cc concentrated HCl and extracted once with 50 cc of ethyl ether in a 300 cc separatory funnel. The ether was washed in the same separatory funnel with 5 cc NaOH (2.5%) and 5 cc dilute HCl. The ether was evaporated over 10 cc water and 5 cc of the water tested for indol by the method given in 5, B. In order to determine the percentage of recovery of indol in the first 100 cc of distillate, a culture tube inoculated with an organism from the pink salmon was incubated for a week and then distilled. Ninety four per cent of the total amount recovered was secured in the first 100 cc. These results indicated that it was not necessary to distill more than 100 cc.

In the summary (table 16) the percentage of indol producers ranges from 0 to 66. The lowest percentages are from bacteria from

TABLE 16. *Summary of indol production by bacteria from raw and canned salmon.*

Source	Species	Months since bacteria taken	No. organisms tested		Indol positive	Skatol positive	—Total— positive	
			No gr'th	Growth			No.	Per cent
1	King	13	3	40	2	1	3	7.2
1	Red	11	10	12	0	0	0	0
1	Coho	11	9	8	0	0	0	0
1	Pink	12	7	25	7	0	7	28.0
1	Chum	10	1	20	9	0	9	45.0
2	All		6	51	9	0	9	17.6
3	All	2	4	36	16	1	17	47
4	King	1		20	10	0	10	50
4	Pink	1		31	17	0	17	54
4	Red	1		24	15	1	16	66
4	Chum	1		9	5	0	5	55
5	Chum	1		23	0	0	0	0
Total				299	90	3	93	31

sources where they have been subjected to unfavorable environment, such as low storage temperatures, long period of cultivation on artificial media, etc. The bacteria in source 5 are those hardy enough to survive cold storage temperatures for three months; apparently all the indol producers were killed by this treatment. The bacteria from the red and coho salmon, source 1, had been on artificial media for 11 months and had been transferred only twice, while those from the other three species had been transferred three times. Several of the cultures were apparently dead, since they gave no growth in Dunham's media. The bacteria found in canned salmon were the survivors of a rather rigorous treatment. The spore-formers are of course the ones most likely to survive hardship, and it would seem that as a class they are not as likely to produce indol as the non-spore-formers. Even under the most favorable circumstances only 66% of the bacteria taken from raw salmon produced indol, while some of those which did not form indol produced a putrid odor. It is therefore easily possible for bacteria to decompose salmon without the formation of indol or skatol. Regarding this, Effront says: "We may conclude from the preceding that the presence of indol is not an infallible characteristic of putrefaction. Numerous aerobes and even certain anaerobes, like *B. putrificus*, which cause the disintegration of albumin to the most simple substances, do not, however, yield phenol or indol. The production of indol indicates merely a mode of attack, a particular direction given to the dismemberment. In other words, it corresponds only to the secretion of an amidase specific for this transformation."

Skatol formation by bacteria was induced (table 16) by only 3 of the bacteria studied in this investigation, when growing in Dunham's media, while 90 formed indol. It is possible that all those bacteria forming indol might, under a different set of conditions, form skatol; but it seems more probable that in those tests in which skatol was formed there was present a specific skatol-forming organism. Such an organism was isolated from culture No. 32 taken from raw king salmon (source 1) and was found to be identical with an organism isolated from canned macaroni. No description of this microorganism has been found in the literature, and it is probably a new species. It is a large, rod-shaped, motile bacterium which, on sporulation, becomes clostridium-shaped, with a large cylindrical spore. It is an obligate anaerobe but has been grown in paraffin-stratified broth in association with a facultative anaerobe from which it has been found rather difficult to separate it. The facultative anaerobe is also rod-shaped, mo-

tile, gram-positive, and spore-forming; it is able to grow in oxygen tensions from almost zero to atmospheric.

B. Formation of indol by scorching proteins

Rohmann (1908) states that both indol and skatol may be formed from tryptophan by heat. Since salmon flesh carries tryptophan, it appeared that scorching might produce indol in perfectly fresh salmon. This had two important bearings on our problem; first, the possibility of indol formation during the distillation in the indol determination, and second, its possible formation during the commercial canning of fish. The first possibility is discussed here, and the second in 7, C. To eliminate the possibility of the formation of indol during its distillation from salmon, the distilling flask was never heated over a free flame but in a bath of nearly saturated salt solution.

To test the effect of scorching on salmon and other proteins, several experiments were carried out among which was the following one. Various substances containing protein were scorched in tin cans, transferred to flasks and distilled in the usual way. The distillates were extracted with ether, which was washed and evaporated. The water test solutions were divided into two parts, one of which was tested with Ehrlich's reagent, and the other with the Vanillin-HCl reagent. The colors produced by the former reagent were extracted four times with chloroform and the residual color noted. The results are given in tables 17 and 18.

TABLE 17. Color produced by Ehrlich's reagent in the indol test on various scorched foods.

Substance scorched	Initial color.	Extractions with chloroform				Residual color equivalent to
		1.	2.	3.	4.	
Peas, canned	Slight; like indol	Pink	Slight	Very sl.	None	1 mmg indol
Beans, canned	Strong; purple; like indol	Pink	Pink	Slight	Very sl.	1 mmg indol
Eggs, fresh	Very strong; purple; like indol	Very strong pink	Strong pink	Pink	Slight	3 mmg indol
Salmon, canned	Strong, purple; like indol	Pink with orange	Violet	Pink	Trace	1 mmg indol
Gelatin, Knox	Strong; more purple than the others	Slight orange	Slight violet	Trace	None	Strong dark purple color; over 20 mmg indol

TABLE 18. *Color produced by the vanillin-HCl reagent in the indol test on various scorched foods.*

Substance scorched	Colors produced
Peas, canned	Weak color; looks about right for indol
Beans, canned	Stronger color than produced in the case of the peas; looks like the color produced by indol
Eggs, fresh	Very strong color; correct for indol
Salmon, canned	Strong color; correct for indol
Gelatin, Knox	Strong color; looks almost like the indol color.

It appears that either indol, or a substance resembling it very closely, is formed by the scorching of those proteins which contain the tryptophan group. Gelatin does not contain this group, but a color is obtained from the scorched gelatin by both the Ehrlich and Vanillin tests which closely resembles the color produced from other scorched proteins which do contain the tryptophan group. However, since the color produced by the Ehrlich test did not extract with chloroform in the case of gelatin, but did in the case of all the other proteins tried, it appears that the color produced from gelatin was not due to indol.

C. *Formation of indol during the processing of salmon.*

Since indol apparently may be formed by scorching salmon, it seemed desirable to determine whether it may be formed during the commercial processing of salmon. This did not seem probable, but in order to be able to state definitely that indol found in cans was the result of bacterial action and not of the cooking process, it was necessary to prove or disprove the possibility of its formation during processing.

A piece of fresh king salmon was obtained and ten half pound (227 g) cans filled. These were tightly closed and immediately placed in the pressure cooker, where they were cooked for 90 minutes at 240° C. The cans were then removed from the cooker and cooled. Eight cans were recooked and cooled as before. The same process was repeated on six cans, then on four, and finally on two. So that cans which had been cooked one, two, three, four and five times, were obtained. Five of the cans representing the five periods of cooking were opened, carefully examined, and the indol determined. The other five cans were later used in the determination of volatile nitrogen. The raw fish used, when tested for indol, gave a negative test. The experiment was repeated, and the results are given in table 19.

The indol is expressed in mmg per 100 g of salmon. Ehrlich's reagent used.

TABLE 19. *Formation of indol during the processing of salmon.*

Experiment	In raw fish	1st cook	2nd cook	3d cook	4th cook	5th cook
1	0.0	0.3	0.6	0.8	1.0	1.3
2	0.1	0.3	0.6	0.6	1.1	1.2

A small amount of indol is apparently formed during the processing of salmon but the amount formed during the usual processing (1st cooking above) is so small as to be practically negligible. The amounts obtained from successive cookings show a gradual and regular increase. The odor varies from normal, through slightly scorched to strongly scorched, while at the same time the fish takes on a scorched flavor. The color grows gradually poorer and the texture softer. The indol color extracted well with chloroform in each case.

D. *Effect of exhaust on the indol content of canned salmon.*

Most of the canned salmon is exhausted before the cans are tightly closed and placed in the retorts. This is usually accomplished by passing the filled cans through a steam box either without the tops or with the tops loosely clinched on. During this process the contents of the can becomes heated, and a part of the air is expelled, resulting in a partial vacuum after the cans are tightly closed, cooked and cooled. The question arises as to the effect of this exhaust on the indol content and the odor of canned fish. Several experiments were carried out, using king, pink and chum salmon in various stages of decomposition. Each fish was cut into sections containing slightly more than one pound (454 g) each. These sections were divided into two equal pieces and each piece placed in a half pound (227 g) can. The cans were marked as usual to show the fish and section, and in addition the cans from one side were marked "E" and those from the other side "N". The covers of the cans marked "E" were loosely clinched and the cans placed in steam at 100° C. (212° F.) for 12 minutes, when the covers were tightly rolled on. The cans marked "N" were tightly closed while the cans were cold. All of the cans were cooked for 80 minutes at 115.5° C. (240° F.)

After the cans had been stored for a few weeks they were examined according to our usual method. The odor of the cans was noted very carefully by three to five men acting independently and without knowing which cans were exhausted and which were not.

The odor of stale and tainted salmon is apparently slightly improved by exhausting the can; for when the separate results of the different examiners were compared it was found in nearly every case that the can in each pair which had been adjudged slightly better in odor was the exhausted can. The indol content in the two cans taken from the same section of the fish, while frequently markedly different, also showed this difference to be as often in favor of the exhausted can as of the unexhausted can. Averages for exhausted and unexhausted cans were nearly equal in most cases. Apparently, therefore, exhausting the can has little effect on the indol content.

8. OTHER DECOMPOSITION CHANGES

An attempt was made to use some of the other decomposition changes and products as measures of decomposition. As stated before, ammonia is without doubt formed from the amino acids during decomposition. However, there is good reason to believe that it may also be formed during the cooking process, creating a doubt as to the origin of the ammonia found in the can. Fatty acids may be formed progressively from fats during decomposition, but it is certain that they may also be formed by heat and pressure. Whether the cooking process in the case of salmon is severe enough to bring about this hydrolysis is a question to be settled by experiment. During the decomposition of salmon a peculiar substance is formed which produces a strong biting sensation when placed on the tongue; it also attacks the skin on the back of the hand. This substance is mentioned in 2. An attempt was made to isolate it.

A. Volatile nitrogen as a measure of decomposition

There are several methods for determining the volatile nitrogen based on distillation or on aeration. The methods used by Loomis (1912) employed distillation from an alkaline medium into standard acid, while Weber (1921) used a modification of the Folin aeration method. The latter method is given by the Assoc. Off. Agric. Chemists (1919) as tentative, and was selected, with certain modifications, for our use. On account of the lack of time, no work was done on the distillation methods. Leach (1920) recommends the use of alcohol in the aerating cylinder, and this was found to reduce the frothing to some extent. The apparatus used consisted of six complete units, so that three determinations in duplicate could be made simultaneously. The air was passed through under pressure, first being

washed in a cylinder containing concentrated sulphuric acid. From the washing cylinder the air passed through six rubber tubes provided with regulating pinch cocks, was conducted by glass tubes to the bottom of the aeration cylinders, and after bubbling up through the mixture of fish and chemicals, was passed through the Folin absorption tube into N/50 H_2SO_4 contained in a 250 cc graduated cylinder. The apparatus, as purchased, provided for the use of bottles four inches (10.2 cm) high for the absorption of the volatile nitrogen compounds; but experiments showed that a much smaller volume of air could be passed through these bottles than through the 250 cc cylinders, and consequently the time of aeration would need to be correspondingly increased.

Twenty five grams of finely ground fish was placed in each aeration cylinder with 150 cc of water, 1 cc of saturated potassium oxalate solution, 25 cc of alcohol, a few drops of phenolphthalein and enough saturated potassium carbonate solution to render the mixture alkaline. Air was passed through as rapidly as possible for six hours and the acid in the absorption cylinder titrated against N/50 NaOH, using sodium alizarinsulphonate as an indicator.

Experiments were now performed to determine whether there was an increase in volatile nitrogen during decomposition, and also whether volatile nitrogenous compounds were formed during the cooking process.

The increase in volatile nitrogen during decomposition was tested by using samples taken from the experimental packs of salmon in various stages of deterioration as described in 6. Of course all of these samples were canned, so when comparing the results it is necessary to assume that if volatile nitrogenous compounds were formed in the canning process the same amount was formed in each of the cans used. The five species were used; the chemical results are given in table 20.

TABLE 20. *Volatile nitrogen in salmon canned at different stages of decomposition.*

Hours out of water when canned	Volatile nitrogen mg per 100 g of fish					
	Pink	Sockeye	Chum	King	Coho	Average
24	17.3	22.3	25.4	34.0	40.9	28.0
48	21.6	19.7	31.7	37.9	38.5	29.9
72	24.3	24.6	35.1	33.8	43.6	32.7
96	32.3	26.1	29.7	37.9	48.4	31.3
120	44.5	41.4	36.6	46.3	55.4	44.8
144	54.3	45.1	47.1	40.4	55.3	48.4

The results show a rather consistent increase in volatile nitrogen from day to day during decomposition in each of the five species. There are a few instances in which the amount decreases; but it must be remembered that a different fish was used for each can, and that some of the fish examined on one day appeared to be in better condition than some of those which were examined and canned the day before. Furthermore, the volatile nitrogen formed during the cooking and during storage must be considered. For the purpose of correlation the amount of indol in these cans was also determined and is given in table 21.

TABLE 21. *Indol in salmon canned at different stages of decomposition.*

Hours out of water when canned	Indol mmg per 100 g of fish					
	Pink	Sockeye	Chum	King	Coho	Average
24	Lost	0.2	0.2	0.1	0.1	0.15
48	"	1.0	1.8	0.8	0.6	1.05
72	"	3.0	4.5	3.3	1.3	3.0
96	"	10.0	16.0	20.0	7.6	13.4
120	"	22.5	52.0	24.0	15.7	28.5
144	45.0	50.0	80.0	22.0	16.0	42.6

Both the indol and the volatile nitrogen increase; but since the former starts from almost zero while the latter starts from an unknown quantity which depends upon the factors of cooking and storage, it is obvious that indol possesses advantages over volatile nitrogen as a measure of decomposition.

The increase in volatile nitrogen during the cooking process was now investigated. Fresh salmon was obtained, the volatile nitrogen determined on a portion, and the rest placed in two half-pound (227 g) cans, one of which was given the usual cooking (80 minutes at 115.5° C., or 240° F.) and the other cooked twice. The volatile nitrogen in each can was then determined. The determinations were made in duplicate. A great deal of difficulty was experienced in the determination of volatile nitrogen in raw salmon on account of excessive frothing. Paraffin oil, kerosene, alcohol and other materials were used, as were various types of baffle plates, but the frothing continued. Although this experiment was repeated several times, only once could results be obtained with raw salmon on account of this frothing. These results are given in table 22.

TABLE 22. *Increase in volatile nitrogen during the canning process.*

Description of sample	Volatile nitrogen, mg per 100 g
Raw salmon -----	11.6
Salmon cooked once-----	22.8
Salmon cooked twice-----	34.0

The results are remarkably uniform and show that cooking splits up the nitrogenous compounds and forms volatile alkaline substances.

Some of the cans which had been packed for the determination of the amount of indol formed in salmon during the canning process (7, C) were used for the determination of volatile nitrogen. These cans had been cooked from one to five times the normal cooking process. Unfortunately no determination of the volatile nitrogen was made in the raw fish; but the indol determination was practically negative, indicating that the fish used was in good condition. The results are given in table 23.

TABLE 23. *Increase in volatile nitrogen during repeated processing.*

Description of sample	Volatile nitrogen, mg per 100 g
Cooked once -----	45.1
Cooked twice -----	45.7
Cooked 3 times-----	56.8
Cooked 4 times-----	57.9
Cooked 5 times-----	64.2

These results show a slight increase for each successive cooking, demonstrating that volatile nitrogenous compounds are split off by it. The amount found after the first cooking is much higher than given in table 22; this difference may be due to the fact that the samples used had been in the cans for nearly a year. Weber and Wilson (1919), when working on canned sardines, found the volatile nitrogenous compounds to increase during storage. This increase might explain why the increase from one cooking to another in table 23 was so much less than in table 22, since more ammonia might be formed during storage in those cans which had the smallest amount at the beginning of the storage period.

The results of both experiments show that the cooking process does increase the volatile nitrogenous constituents of salmon. The

results also tend to confirm the report of Bidault and Couturier (1920) that the amount of ammonia in canned meat is a function of the heat of sterilization.

B. Increase in free fatty acids as a measure of decomposition in salmon.

Weber (1921), in his work on the Maine sardine, determined the fat and the free fatty acids in sardines which had been held in brine for periods ranging from 2 to 96 hours, and concluded that no change of a significant nature was shown by the results. However, we made a few experiments, none of which gave satisfactory results.

The first method used was based on that of Folin and Wentworth (1910) for the determination of fat and fatty acids in feces. The cans of salmon used were opened, carefully examined, and then thoroughly mixed. Ten gram portions were weighed out on lead dishes and dried in a vacuum oven at 80° C., cooled and weighed. The lead dishes containing the dried salmon were then placed in Soxhlet extractors and extracted for 16 hours with anhydrous ether containing sufficient anhydrous HCl to make the ether solution approximately tenth normal. The ether was distilled from the flasks and petroleum ether added. After standing over night the petroleum ether solution was filtered into a weighed flask and the residue washed with petroleum ether. The petroleum ether was then evaporated; the residue weighed, dissolved in benzene, and titrated with N/10 sodium ethylate, using phenolphthalein as an indicator.

This method did not prove satisfactory and the experiments were repeated using anhydrous ethyl ether instead of the ether-HCl solvent.

In the investigation of the free fatty acid in salmon canned at different stages of decomposition, the cans used were part of the experimental pack of king salmon described in 6, B. Two portions of each were taken from each can, one portion (*a*) was extracted with the ether-HCl solvent and the other (*b*) with ether alone. The results are expressed as milligrams of stearic acid per gram of fat in table 24.

The results for solids are uniformly higher in the (*b*) samples than in the (*a*) samples; this is due to the fact that the (*b*) samples were secured after the ground fish had been standing exposed to the air for an hour and had apparently lost some of the moisture. The fat is also higher in most of the (*b*) samples. The results for fatty acids are very contradictory. The results for the (*a*) samples are higher than those for the (*b*) samples, except in the case of K2, and

TABLE 24. *Solids, fats, and fatty acids, in king salmon canned at different stages of decomposition.*

Can No.	Hours out of water before canning	Solids, per cent	Fat, per cent	Fatty acids as stearic acid mg per g fat
A2	24	(a) 39.70	17.00	114.2
		(b) 39.89	17.25	74.0
D2	48	(a) 39.96	14.63	102.9
		(b) 40.41	16.92	37.7
G2	72	(a) 44.65	24.50	135.5
		(b) 45.12	21.15	70.0
K2	96	(a) 39.56	17.16	93.7
		(b) 40.24	17.81	113.2
N2	120	(a) 38.67	15.55	95.2
		(b) 39.90	16.32	70.4
P2	144	(a) 38.67	13.74	115.2
		(b) 39.44	14.22	96.9

suggest two possibilities; either the acid in the ether-HCl solvent partially hydrolyzed the fat, or else the HCl was not entirely removed when the ether was evaporated. Even the (b) samples, with which no HCl was used, do not show a consistent increase during decomposition. The method appears to be of little value as a measure of decomposition in canned salmon.

In the investigation of the free fatty acid in salmon before and after canning, portions of a fresh king salmon were used. The solids, fats and fatty acid were determined both before and after canning. Anhydrous ether was used for extraction. The results are given in table 25.

TABLE 25. *Free fatty acid in salmon before and after canning.*

Description of sample	Solids, per cent	Fat, per cent	Fatty acids as stearic acid mg per g fat
Raw salmon -----	25.64	5.36	47.6
	25.64	5.31	23.8
Canned salmon -----	31.95	9.18	13.9
	31.94	9.11	14.0

The results are not favorable to the theory that the fatty acid is

increased during the canning process, but the data is of course too meager to warrant the drawing of definite conclusions.

The amount of free fatty acid was also determined in some king salmon which had been cooked one, three and five times. These cans were a part of those described in 7, C. The results are given in table 26.

TABLE 26. *Effect of cooking on the free fatty acid content of canned salmon*

Description of sample	Fat, per cent	Fatty acids as stearic acid mg per g fat
Cooked once -----	7.94	173.5
Cooked 3 times-----	8.24	170.5
Cooked 5 times-----	6.45	205.8

The results do not show a satisfactory correlation between the number of times cooked and the amount of free fatty acid.

The three experiments described above indicate that the determination of the free fatty acid is not likely to be of value in detecting decomposition in canned salmon.

C. *Formation of a substance having a biting taste.*

Tainted canned salmon, when placed on the tip of the tongue, produces a sensation similar to that produced by "strong" cheese. If the salmon be rubbed on the back of the hand the skin becomes irritated and itches. Canned salmon in good condition does not produce these sensations. Some new substance has been formed during the decomposition. The same substance, or at least one giving the above sensations, is found in partially decomposed tuna. In both cases its presence appears to be associated with "honey-combing." Several attempts were made to isolate the substance; but although it was obtained in a highly concentrated form, as judged by the effect on the tongue, it was never obtained in a pure state.

9. SUMMARY

The canning of salmon constitutes one of the most important industries of the Pacific coast of North America. Salmon, in common with other fish, are delicate, easily injured and very easily decomposed; and there are many opportunities for spoilage between

the time they are taken from the water and the time they are canned. A systematic method for the examination of canned salmon has long been needed, and such a method is herein outlined.

The literature on the chemical composition of fish flesh is reviewed. Original data are given, with food value, based on the analysis of 643 cans of salmon comprising individual fish of the five species from each important canning district. The decomposition of fish flesh together with the utilization of certain decomposition products as a means of estimating the amount of spoilage is discussed. Indol and skatol are the products finally selected as the most suitable for this purpose.

The principal color tests for indol and skatol are given; the Ehrlich, Herter and dimethylaniline tests are chosen as the most sensitive. These three tests are modified and improved and their delicacy increased; a method for the determination of indol and skatol in salmon is developed.

A biochemical study is made of the five species of salmon, covering the physical and bacteriological changes, and the appearance and increase of indol and skatol during progressive decomposition. Data regarding the 138 salmon studied along each of the above lines of investigation are given in tables showing the average daily decomposition changes, and in curves showing the increase in indol. Skatol was not found in any of the raw salmon, or in the experimental cans prepared from them; but was found in several commercial cans of salmon. An organism capable of forming skatol in salmon flesh was, however, isolated from one of the raw king salmon. Indol was found in small amounts in each of the salmon examined at the end of 48 hours storage, but only three of them contained more than 1.5 mmg per 100 grams. In general it may be said that when this quantity of indol is found in canned salmon a considerable degree of decomposition has taken place.

Indol was quantitatively determined in 544 commercial cans of salmon. As some of these cans had a strong tainted odor and yet contained very little indol, the absence of indol cannot be taken as complete evidence that decomposition has not taken place. The experimental or laboratory packs of salmon contained more indol than commercial packs; all experimental cans classed as tainted contained more than 1.5 micromilligrams per 100 grams.

Rather close correlation was found between the number of bacteria present from day to day during spoilage and the indol content; this is shown by means of curves. A close correlation was

also found between the storage temperature and the indol content; king salmon stored at the highest temperature had the most indol, while the sockeye and coho salmon stored at the lowest temperature had the least.

The gills were found to have more bacteria and more indol than either the viscera or flesh. The flesh was sterile at the end of 24 hours in most of the fish examined, and contained little or no indol. The cooked flesh appeared to contain slightly more indol than the raw, but this may be due to the action of the cooking process in breaking down the cellular structure of the fish, resulting in a more rapid and complete liberation and distillation of the indol. The first cut of the fish, just behind the gills, was found to contain more indol than a cut through the middle of the fish in the region of the dorsal fin.

Exhausting the cans of stale and tainted fish was found to improve slightly their odor, but there was apparently no change in the indol content. Indol, or a substance very closely resembling it, is apparently formed by the scorching of those proteins, including salmon flesh, which contain the tryptophan group. A small amount of indol may be formed during the processing of salmon, but this amount is so small as to be negligible. Excessive cooking produces slightly more indol, a scorched odor and flavor, and a slightly softer texture.

The indol producing power of 299 different cultures of bacteria taken from raw or canned salmon was tested and only 31 per cent gave positive tests. The comparatively small number of these bacteria which produced indol, suggests a reason why so large a percentage of the tainted commercial cans contained little or no indol.

The canning process appears to increase the amount of volatile nitrogen (ammonia and amines) in salmon. The volatile nitrogen content of salmon increases from day to day during decomposition with considerable regularity; but owing to the probability of its formation during the cooking process, it is not suitable as a measure of decomposition in canned salmon, although no doubt it is of value as a criterion in the case of raw salmon.

Very unsatisfactory results were obtained in attempting to use the free fatty acids of salmon oils as a measure of decomposition of the fish from which they were extracted. Tainted canned salmon, when placed on the tip of the tongue, produces a sensation similar to that produced by "strong" cheese. A substance producing this sensation was obtained in a highly concentrated but impure form,

and the attempt to identify it was abandoned on account of lack of time.

In conclusion it may be said that although the determination of indol cannot supplant odor and physical appearance in the examination of canned salmon, it is nevertheless of value and affords considerable information as to the previous history of the sample.

10. BIBLIOGRAPHY

1822. Morin. Examen chimique de l'Eperlan, *Salmo eperlanus*. Jour. Pharm. 8:61.
1847. Liebig, J. Ueber die Bestandtheile des Flüssigkeiten des Fleisches. Ann. 62:257-369.
1854. Payen, A. Precis des substances alimentaires. Polyt. Jour. 134:385.
1874. Buckland, F. Bestandtheile des Fleisches von Lachsen und Heringen im Vergleich zu dem von Rindfleisch. Arch. Pharm., series 3, 53:178.
1876. Koenig, J. Der Gehalt der Menschlichen Nahrungsmittel an Nahrungstoffen im Vergleich zu ihren Preisen. Zeitsch. Biol. 12:497-512.
1883. Salkowski, E. Zeitsch. physiol. Chem. 8:425.
1885. Bocklisch, O. Ueber Faulnisbasen (Ptomaine) aus Fischen. Ber. 18:1922-1927.
1887. Almen, A. Analyse des Fleisches einiger Fische. Mitgeteilt der Kgl. Ges. der Wissenschaft, Upsala. Pub. by E. Berting.
1888. Atwater, W. O. The chemical composition and nutritive value of food fishes and aquatic invertebrates. Rept. U. S. Comm. Fish and Fisheries.
1893. Fahrion, W. Contributions to the knowledge of train oils. Jour. Soc. Chem. Ind. 12:935; from Chem. Ztg. (1893) 684-686.
1897. Moerner, C. T. Ueber ein eigentümliches Nahrungsmittel, nebst einiger Beobachtungen über darin angetroffene Faulnisbasen. Zeitsch. physiol. Chem. 22:514.
1898. Langworthy, C. F. Fish as food. U. S. Dept. Agric., Farmers Bull. No. 85.
1903. Hopkins, F. G. and Cole. Constitution of tryptophane and the action of bacteria on it. Jour. Physiol. 29:451.

1903. Rettger, L. F. An experimental study of the chemical products of *Bacillus coli communis* and *Bacillus lactis aerogenes*. Amer. Jour. Physiol. 8:284.
1905. Herter, C. A. On a relation between skatol and the dimethylamidobenzaldehyde (para) reation of the urine. Jour. Biol. Chem. 1:251.
- 1905a. Herter, C. A. (A note on the separation of indol and skatol). Jour. Biol. Chem. 1:255.
1905. Herter, C. A. and Foster, L. A method for the quantitative determination of indole. Jour. Biol. Chem. 1:257.
1906. Dakin, H. D. The glyoxylic acid reaction for tryptophan, indol and skatol. Jour. Biol. Chem. 2:289.
1906. Escallon, J. and Sicre, A. Reserche de l'indol dans les cultures microbiennes a l'aide du furfural. Compt. rend. soc. biol. 65:507-508.
1906. Herter, C. A. and Foster, L. On the separation of indole from skatole and their quantitative determination. Jour. Biol. Chem., 2:267-272.
1906. Konto, K. Ueber eine neue Reaktion auf Indol. Zeitsch. physiol. Chem. 48:185.
1906. Rettger, L. F. Studies on putrefaction. Jour. Biol. Chem. 11:71.
1906. Steensma, F. A. Ueber Farbenreaktion der Eiweisskörper, des Indols und des Skatols mit aromatischen Aldehyden und Nitriten. Zeitsch. physiol. Chem. 47:25.
1906. Tobey, E. N. The methods for testing the indol reaction. Jour. Med. Res. 15:301-304.
- 1906a. Tobey, E. N. The cholera-red reaction and the indol reaction. Jour. Med. Res. 15:305-308.
1908. Deniges, G. Cinnamic aldehyde and vanillin as reagents for indol. Repert. pharm. (3) 20:161; from Bull. soc. pharm. (Bordeaux).
- 1908a. Deniges, G. Detection of indol by the reactions of Ehrlich and of Legal. Repert. pharm. (3) 20:160; from Bull. soc. pharm. (Bordeaux).
1908. Knisely, A. L. Composition of canned salmon. Pac. Fisherman 6:21.
1908. Morelli, E. A new method for identifying indole in bacteriological work. Rif. med., No. 5; through Biochem. Centr. 8:286

1908. Osborne, T. B. and Heyl, F. W. Hydrolysis of fish muscle. *Amer. Jour. Physiol.* 23:81-89.
1908. Rettger, L. F. Further studies on putrefaction. *Jour. Biol. Chem.* 4:45.
1908. Röhmann, F. *Biochemie.* p. 445. Julius Springer, Berlin.
1908. Von Moraczewski, W. Ueber eine Methode der quantitativen Indolbestimmung im Kote. *Zeitsch. physiol. Chem.* 55:42.
1909. Blumenthal, F. Tests for indole and skatole. *Biochem. Zeit.* 19:521; through *Chem. Zentr.* 2:865.
1909. Ehrlich, F. Entstehung der Bernsteinsäure bei der alkoholischer Gährung. *Zeit. Ver. deut. Zucker-Ind.,* p. 646.
1909. Koenig, J. and Splittgerber, A. Die Bedeutung der Fischerei für die Fleischversorgung im Deutschen Reich. *Landw. Jahrb.* 38:1-169.
1909. Suwa, A. Untersuchungen über die Organextrakte der Selachier. I. Die Muskelextraktstoffe des Dornhais (*Acanthias vulgaris*). *Arch. ges. Physiol.* 128:421-426.
1909. Suzuki, U. and Yoshimura, K. Ueber die Extraktivstoffe des Fischfleisches. *Zeitsch. physiol. Chem.* 62:1-35.
1910. Folin, O. and Wentworth, A. H. A new method for the determination of fat and fatty acids in feces. *Jour. Biol. Chem.* 7:421-426.
1911. Effront, Jean. Sur la fermentation putride. *Moniteur Scientifique Queaneville.* p. 489.
1912. Loomis, H. M. The salmon canning industry of North America. *Orig. Commun., 8th Intern. Cong. Appl. Chem.* 18:239-245
1912. Okuda, Y. Quantitative determination of creatin, creatinin, and monoamino acids in some fishes, mollusca and crustacea. *Orig. Commun., 8th Intern. Cong. Appl. Chem.,* 18:275-281.
1912. Van Ermengem, E. Der *Bacillus botulinus* und der Botulismus. In Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen.* 2nd ed. Jena. 4:909.
1913. Yoshimura, K. and Kanai, M. Beiträge zur Kenntniss der stickstoffhaltigen Bestandtheile des getrockneten Kabeljau. *Zeitsch. physiol. Chem.* 88:346-351.
1914. Lewkowitsch, J. *Chemical technology and analysis of oils, fats and waxes.* 5th ed., Vol. II. MacMillan and Co., London.

1914. Schoendorff, B. and Wachholder, K. Ueber den Glykogenstoffwechsel der Fische. I. Der Glykogengehalt van Süßwasser-fischen. Arch. ges. Physiol. 157: 147-164.
1915. Baudisch, O. Ueber eine neue Indolreaktion. Zeitsch. physiol. Chem. 94:132-135.
1916. Elliott, S. and Clemens, C. A. Composition of canned salmon. South Dakota Food and Drug Dept. Bull., Vol. 4, Nos. 2 and 3, p. 8-11.
1916. Nelson, V. E. Some color reactions for indole and skatole. Jour. Biol. Chem. 24:527.
1917. Bergheim, Olaf. The determination of fecal indol. Jour. Biol. Chem. 32:17.
1917. Effront, Jean. Biochemical catalysts in life and industry. Translated by S. C. Prescott. John Wiley and Sons, New York.
1917. Tanner, F. W. Studies on the bacterial metabolism of sulphur. Jour. Bact. 3:565-593.
1918. Clark, E. D. and Almy, L. H. A chemical study of food fishes. Jour. Biol. Chem. 33:483-498.
1918. Hawk, P. W. Practical physiological chemistry. 6th ed. P. Blakiston's Son and Co. Philadelphia.
1919. Assoc. Off. Agri. Chem. Methods of analysis. p. 210.
1919. Green, C. W. Biochemical changes in the muscle of the king salmon during the fast of spawning migration. Jour. Biol. Chem. 39:435-456.
1919. Okuda, Y. Chemistry of "chiai" flesh. Jour. Coll. Agric. Imp. Univ., Tokyo, 7:1-28.
- 1919a. Okuda, Y. Extractive matters of *Palinurus japonicus* and *Loligo brekeri*. Jour. Coll. Agric. Imp. Univ., Tokyo, 7:55-67.
1919. Okuda, Y., Okimoto, T. and Yada, T. Hydrolysis of the muscle proteins of the whale and the cod. Jour. Coll. Agric. Imp. Univ., Tokyo, 7:29-37.
1919. Okuda, Y., Uematsu, S., Sakata, K., and Fujikawa, K. Hydrolysis of the muscle proteins of *Loligo brekeri*, *Palinurus japonicus* and *Paralithoides camtschatica*. Jour. Coll. Agric. Imp. Univ., Tokyo, 7:39-54.
1919. Tanner, F. W. Bacteriology and mycology of foods. John Wiley & Sons, New York.
1919. Weber, F. C. and Wilson, J. B. The formation of ammonia and amines in canned sardines during storage. Jour. Ind. Eng. Chem. 11:121.

1920. Am. Pub. Health. Assoc. Standard methods of water analysis.
1920. Benson, C. C. Urea in dogfish and skate. *Jour. Biol. Chem.* 41:60.
1920. Bidault, C. and Couturier, G. The action of heat on the vitamins of meat. *Compt. rend. soc. biol.* 24:1022.
1920. Houghton, H. W. and Hunter, A. C. The decomposition of salmon. *Pacific Fisherman* 18: No. 5, p. 38.
1920. Ingvaldsen, T. and Bauman, L. Note on the preparation of para-dimethylaminobenzaldehyde. *Jour. Biol. Chem.* 41:145.
1920. Leach, A. E. Food inspection and analysis. 4th ed., p. 226. John Wiley & Sons, New York.
1920. Zoller, H. F. Quantitative estimation of indole in biological media. *Jour. Biol. Chem.* 41:25.
- 1920a. Zoller, H. F. Influence of hydrogen ion concentration upon the volatility of indole from aqueous solution. *Jour. Biol. Chem.* 41:37.
1921. Beal, G. D. and Brown, J. B. A study of the highly unsaturated fatty acids occurring in fish oils. *Science (New Series)*, 54:175.
1921. Bigelow, W. D. and Cathcart, P. H. Relation of processing to the acidity of canned foods. *Nat. Can. Assoc. Res. Lab. Bull.*, 17-L.
1921. Cobb, J. N. Pacific salmon fisheries. *Bur. Fish. Doc.* 902:152.
- 1921a. Cobb, J. N. Pacific salmon fisheries. *Bur. Fish. Doc.* 902:143.
1921. Dill, D. B. A chemical study of certain Pacific Coast fishes. *Jour. Biol. Chem.* 48:73-82.
1921. Weber, F. C. The Maine sardine industry. *U. S. Dept. Agric. Bull.* 908:91.
- (?) Barton, A. W. and McMillan, F. A. Work on the diastatic enzymes of the salmon. Unpublished. Department of Chemistry, University of Washington.
- (?) Clark, E. D. and Shostrom, O. E. Composition of Pacific Coast salmon. Unpublished. Lab. Nat. Cannery Assoc., Seattle.
- (?) Pugsley, W. H. Decomposition in salmon before and after cold storage. Unpublished. College of Fisheries, University of Washington.
- (?) Sasaki, T. A new delicate skatole reaction. *Biochem. Zeit.* 23:402-403.
- (?) Weehuizen, F. Indole in flowers. *Pharm. Weekblad.* 45:1325-1329.

LIBRARY OF CONGRESS



0 014 423 011 5